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National Institute On Aging.  
Report of program activities.



NATIONAL INSTITUTE ON AGING

ANNUAL REPORT

JULY 1, 1976 THROUGH SEPTEMBER 30, 1977





# NIA ANNUAL REPORT

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NIA ANNUAL REPORT

Office of the Director

1. Office of the Director

OD-01

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OD/IO-22



NIA ANNUAL REPORT  
JULY 1, 1976 THROUGH SEPTEMBER 30, 1977  
OFFICE OF THE DIRECTOR

Introduction

The National Institute on Aging was established "for the conduct and support of biomedical, social, and behavioral research and training related to the aging process and the diseases and other special problems and needs of the aged."

Those words from the Research on Aging Act of 1974 state in brief the mission of the National Institute on Aging. Although the Act officially established this eleventh of the National Institutes of Health in 1974, it was not until mid-1976 that the Institute became truly operational. We are still a young Institute, but much has been accomplished in our first year--with emphasis on coordination, planning and development, as might be expected in a new venture.

The broad mandate of the Institute, the wide spectrum of its scientific and social concerns, and the previous lack of a federal focal point for the study of aging demanded that a firm base be established. We resisted the temptation to move too rapidly along the many avenues open to exploration and action. A two-fold approach was taken: (1) support for a continuation of work in progress,\* with some modifications and expansions, and (2) careful preparation of a blueprint for the future.

An analysis and clear definition of our mission was necessary, as was the setting of priorities. That basic work has now largely been done, most notably in a master plan for DHEW research into aging that was presented to Congress in December, 1976. That document, which details priorities and long- and short-range goals, is entitled "Our Future Selves: A Research Plan Toward Understanding Aging." In the introduction to the plan the fundamental goal of the Institute was stated:

"The quality, not alone the quantity, of life should be the quintessential goal of research as well as other human efforts -- lest our future selves will be in jeopardy."

Part of the development of the plan involved preparation of Panel Reports on social and behavioral sciences, service delivery, and biomedical aspects of aging. These constitute a useful state-of-the-art compendium on gerontology and geriatric medicine in the United States.

\*The core of NIA's research programs was drawn from the National Institute of Child Health and Human Development.

The end of the initial period of operation marks the Institute's movement from preliminaries and preparation toward new activities designed to accomplish the objectives of the Research on Aging Act.

Our ultimate purpose is, really, to contribute to the amelioration of suffering in old age through the application of research discoveries. To my mind, NIA is extremely fortunate to be part of the National Institutes of Health, with broad opportunities for interaction and collaboration in the study of a universal biological state--aging--and the specific diseases that are the focus of interest of other Institutes.

While keeping in mind that research is the primary function of the Institute, practical considerations are an important part of all planning and development. In order to improve the quality of life by extending the healthy, productive middle years, it is essential to obtain new knowledge and to transmit that knowledge plus what is already known to our constituencies. Research and education can contribute much to enhanced health care and service delivery.

Cost containment is also a major concern. In establishing priorities, the Institute considers whether proposed studies will contribute to the understanding or resolution of a particular problem. we also attempt to determine the costs involved, the personal and family anguish that might be lessened, and whether research in an area will enhance the quality of life.

At the same time, we attempt to make certain that concern about costs does not tip the balance against our equally important

objective of quality. We seek sound research which is, literally, the ultimate service and the ultimate cost-container.

#### A Joint Venture

A workshop-conference on Alzheimer's disease/senile dementia and related disorders of the brain illustrates the Institute's triple concerns--sound research, practicality, and collaboration. The conference was co-sponsored by the NIA, the National Institute of Neurological and Communicative Disorders and Stroke, and the National Institute of Mental Health.

There are some 1.2 million patients in costly long-term care institutions and it is estimated that as many as 65 percent of all older patients are in these homes because of brain disorders. Clearly, understanding the causes of organic brain diseases and identifying the many reversible conditions that may mimic them would do much to alleviate the financial and emotional burdens of placing old people in nursing homes.

Out of this conference grew reports of three commissions concerned with the field of brain disorders. There will be a followup among the three Institutes concentrating on psychosocial factors and behavioral aspects of senile dementia, as well as service delivery. The aim is to give a full accounting of the status of research on and service to those suffering from this major affliction--and to base future program planning and policy decisions on that foundation.

Similar joint conferences and programs have been--and will be--conducted as the Institute takes full advantage of the opportunities



for interaction growing out of our concern with the universal biological state of aging and the fact that our interests lie within and across disciplines which are the focus of categorical Institutes.

These collaborative efforts have extended beyond NIH to other governmental and non-governmental agencies and organizations with knowledge and interests which fall into the broad framework of our mandate. We keep in mind the fact that great bodies of information already exist, needing only to be tapped, extended, or refocused; it is not necessary to reinvent the wheel. Aging is a lifelong process and virtually all subjects of medical interest are of concern to NIA, from the developmental diseases of children through the many cycles of life that culminate in old age.

#### Improvement of Life

There are at least three basic ways in which we can improve the lives of older people. The first is improvement of the socio-economic conditions under which they live. In 1974, the median income was \$69 per week per individual--less than \$10 a day. Those are absolute figures but it is quite apparent that the official poverty indices are not realistic, not meaningful expressions of how people can live adequately.

Another route to improvement is, of course, research. More extensive comment on this topic follows.

A third, very vital approach to enhancing the quality of life in the later years is through a change in attitudes, considered

in terms of three concepts: (1) prejudices--the negative attitude toward the old called "ageism;" (2) denial of the fact of aging; and (3) a sense of futility.

As for ageism, our language all but creaks from the weight of negative terminology in regard to older people. Such terms as "crock" and phrases like "out to pasture" are epithets that no racial or ethnic minority would accept, yet we hear them daily in reference to older people. There is massive evidence of personal and institutionalized prejudice toward people simply because they become older. They are seen as frail, dependent, sexless, boring and unproductive. Overlooked are the positive aspects of aging--the gathering of wisdom and experience to share, the physical and mental vigor of many older people well into the later years.

Another harmful attitude is denial of our own aging. Most Americans are quite frightened of dying and death and pretend that it won't happen. Clearly, we won't do anything about those matters we avoid facing and, therefore, denial invites and encourages a feeling of futility.

### Statistics

Many are familiar with the figures, but they bear repeating so that they are neither forgotten nor ignored. The striking demographic picture of the 20th century shows that by the year 2030, at least 17 percent of the American population will be 65 years of age or older--that compares with only four percent of the population in that age group in 1900. These projections are based upon the reality of a population that already exists. The figures on the growth of

the older population reflect the post-world War II "baby boom"-- those people who were reported during the 1960's as busy at the greening of America are now taking part in the graying of America.

Those projections are conservative because they are based upon a continuation of the present health care delivery system. If there are improvements, the percentage of older people should increase. The projections are also based on present biomedical knowledge so, obviously, any major discoveries would contribute to a further increase in numbers.

It seems reasonable, given the fact that past population projections have underestimated the survivorship of older people, to expect that close to 20 percent of the population will be aged 65 and older by 2030. This means that our institutions have only 50 years of lead time to prepare to meet the needs--and demands--of this large segment of society. These are statistical facts and they are impressive. Equally important are truths that have grown with and, at times, have overshadowed these facts.

### Study of Aging

The nation's elderly hold up a distorted mirror to society, a distortion imposed by two elements: the vital supports which many of the elderly lose merely by the fact of having become aged; and our ignorance of what aging is--an ignorance made the more profound by societal resistance to acknowledging the fact of aging and preparing adequately for it. Accordingly, two premises underlie the recommendations of the NIA plan for research:

- Effective programs for the aged--whether medical or social, treatment or prevention--must be based on knowing which changes in the aged are intrinsic to the aging process and which are not.
- If we are able to prevent or lessen the impact of non-intrinsic factors in the decline of the aged, then we are left with a unique and more satisfying concept of what aging is or should be--a natural stage in human development leading to a gradual and peaceful end.

Throughout our work, we strive to distinguish the normal aging process from the diseases that may accompany aging, and to mount effective programs of research which differentiate between changes due to the normal process of aging and those due to an individual's heritage, lifestyle and condition of living.

We continue, of course, to investigate the diseases and ailments that are especially common or present differently in the old. These include: drop attacks, decubitus ulcers (bedsores), incontinence, pain, and episodes of confusion. In the area of endocrinology, we will initiate clinical trials of estrogens in the post-menopausal period and study effective means to prevent and treat hypertension.

#### Cost Containment

Throughout our studies, it is always remembered that hard-won knowledge must be applied to practical ends. NIA must devote its resources, for example, to the effort to curb the incredibly staggering costs associated with the debilities of old age. Growing costs erect barriers to the delivery of the benefits of knowledge we already possess to far too many people of all ages, not only the elderly. For this reason, cost containment is a legitimate goal of research.

### Present Tasks

With these fundamental considerations in mind, the Institute has gone on to implement an active program of research.

We are recruiting a Deputy Director who will be concerned primarily with coordination. It is crucial, for example, that NIA coordinate effectively with such institutions as the Veterans Administration, the other National Institutes of Health, and the National Center for Health Statistics. We also now have on staff an analytic epidemiologist as Associate Director who will provide long-term planning and a scientific basis for any kind of preventive medicine program. The Special Projects Officer will deal with the many significant issues that arise, ranging from the protection of elderly research subjects to a critical and effective response to a variety of public policy issues.

We have been engaged during this first year in what we call the necessary capitalization of the Institute to make it fully operational. The excellence and dedication of the present staff have been rewarding to me, having come from the private sector. Although I have despaired on occasion at the length of time it takes for recruitment, I am extremely pleased with the key people we have found so far.

Planning has, of course, been a major activity. NIA's growth should proceed reasonably and only as research training needs and opportunities arise. We are not engaged in building an empire but in playing a major coordinative, catalytic and supportive role in

the development of programs that will lead to new knowledge for understanding and treating major conditions. For example, we will study senile dementia, which has been estimated to afflict more than half of the 1.2 million Americans in nursing homes, and osteoporosis, which afflicts at least 14 million post-menopausal women.

We are exploring all possible ways for collaborative planning and funding of quality research with other relevant federal agencies. we are now working on guidelines for the NIH Division of Research Grants. We may expand them for publication in various journals because they describe the legitimate scientific domain of aging research.

#### Priorities

As to the setting of priorities, we are seeking to preserve investigator-initiated research throughout the academic community. within the intramural program of NIA, we insist on an intellectual basis for deciding that certain priorities are appropriate and can be put in some kind of rank order. Our criteria for establishing priorities include:

- Personal and family anguish. The loss of physical and mental capacities and the fear of "being put away" are two of the most frightening specters of old age.
- Social cost. Currently, over half of the federal health dollar is spent on older people.
- Readiness of topic for understanding through research.  
An excellent example is the paradoxical effects of some drugs in older individuals.

- Program balance. The law that created the NIA clearly and wisely suggests the need for a complex response to a complex problem. It is not one that can simply be seen in terms of a single discipline or a single profession. The law specifically directs us to study social and behavioral aspects as well as biomedical aspects of old age. We don't yet have that kind of program balance in the research grants that have come forth.

- Infrastructure, or mechanism of the research enterprise.

The development of a cadre of trained investigators and teachers is especially critical. That group will live long beyond any of us because once you have established trained manpower and the basic resource of populations for study, the effort extends far beyond any particular topic of study and also sets the stage for future research.

#### Drugs and Prosthetics

In the area of pharmacology, we don't need new concepts or new instruments in order to achieve better understanding of drug-age, drug-food, and drug-drug interactions and incompatibilities.

Another short-term effort is appropriate in the application of modern technology to the development of prosthetic devices. Our efforts toward collaboration in the area of prosthetics have had considerable support here at NIH, and collaboration with the National Aeronautics and Space Administration is under way to develop prosthetic devices such as actuators, manipulators and sensors to aid individuals of all ages who have sensory and motor losses. We are also discussing

this subject with representatives of the National Bureau of Standards and look forward to cooperation with the Veterans Administration, which already has a well-developed prosthetic program.

A workshop in pharmacology was held in September. It brought pharmacologists who have not been in the field of aging together to elicit their interest in developing research proposals regarding the pharmacology of aging, pharmacokinetics, absorption and distribution, pharmacodynamics, and behavioral toxicity of drugs. We have contracted with the Boston Collaborative Drug Surveillance Program to collect data on the responses of older people to drugs. This will provide material that will help in the creation of prescription guidelines for physicians working with older people.

#### Baltimore Longitudinal Study of Aging

The Baltimore Longitudinal Study of Aging at the Gerontology Research Center enters its twentieth year in 1978. This study of normal aging has examined more than 1,000 men ranging in age from 20 to 90 over the past two decades and continues to produce findings of value to a broad range of scientists working in the field of aging and human development, as well as those investigating specific diseases.

Women will be added to the study in January, 1978, an obviously important addition that will make the ongoing research truly comprehensive. For example, we need to learn why women outlive men by an average of eight years. Since they tend to be three years younger than the men they marry, American women generally can expect 11 years of widowhood. As a result, among the 23 million older Americans



today, over thirteen million are women and just under nine million are men. These facts alone have important implications for economists and social scientists as well as those in the medical profession.

The Baltimore study may turn out to be the first family study of this kind. The added women are for the most part wives and daughters of present volunteers; this may provide opportunities for cross-validation of independently derived autobiographical reports. We will examine cyclicity of women, clinical immunological differences, and the differential bone density between the sexes.

We need also to compare the concepts, methods and findings of other longitudinal studies, such as the Framingham, Duke, and Boston VA studies. They constitute a national resource.

This past year, we convened a conference on autopsy which brought together pathologists, gerontologists and representatives of longitudinal studies. Out of that meeting came a task force to handle the legal, logistical and scientific arrangements for obtaining autopsy material from participants who dropped out of the Baltimore study.

By widely disseminating findings from this and other studies, we hope to help reduce the frequency of misdiagnosis, misplacement and mismanagement of the elderly patient, with a corresponding reduction in the cost of health care. Information and education must not be limited to health care providers, but should reach older people and their families. Family and self-care, particularly in the area of health incentives or deterrents, can have preventive as well as curative results.

## Research Areas

The foundation of the NIA research program is the biology of aging--cell biology--and it is a major priority of our programming. To indicate the Institute's broader range of research interests and its non-categorical nature, some areas of special emphasis include:

- The relationships between drugs and age
- The medical and emotional impact of retirement and assessment techniques for determining functional capability in a flexible retirement system
- The nutritional needs of the aged
- Organic brain diseases
- Immunological decline with age
- Differences in life expectancy between the sexes among racial and ethnic groups
- The development of prosthetic devices
- Preventive medicine
- Physical fitness and aging
- Sleep disturbances in old age
- Endocrinology
- Immunology
- Neurosciences
- Neurobiology
- Animal and biological resources
- Epidemiology
- Clinical trials and various longitudinal studies

Some might consider this too broad and lengthy an agenda. we recognize that we cannot support each and every area to the full measure, but we do intend to think through and begin the developmental process of each subject area. Given the complexity and breadth of our mandate, I think this is probably as reasonable and sensible a research prospectus as one could come up with at this particular time.

### Geriatric Medicine

In order to make advances in the study of aging and to reap the benefits, it is necessary to have a cadre of trained professionals in geriatric medicine--the practice and teaching of which is in a sorry state in the United States. Geriatric medicine continues to be a serious concern, but signs of progress have begun to appear in the past year.

There are no more than ten truly outstanding geriatricians in the nation; most schools of medicine do not even have electives in the subject, not to mention curricula, and at present there are only two professorial chairs in the field at major universities. we have a body of knowledge in geriatrics and gerontology but it is neither properly assembled nor is it being taught. we are in a chicken-and-egg situation: now are we to develop the needed new geriatricians when we do not have the people to encourage and train them?

Some encouraging signs of change:

The NIA has contracted with the Institute of Medicine of the National Academy of Sciences to select a group of outstanding

people from medical academia to evaluate the best means of introducing material regarding human aging into medical school curricula. The objective of the IOM project is to determine how best to provide the necessary instruction.

Interest among medical students was indicated at a workshop of the American Medical Student Association attended by the Director last fall. This one brief meeting proved so popular that the organization decided to devote a full session to geriatric medicine at its annual meeting.

Continuing contact in this area is maintained with the Veterans Administration, which responded favorably to our suggestion that its fellowship program be used to send physicians to study in the United Kingdom, where there are ten chairs in geriatric medicine. We are hopeful that the VA Fellowship Program, along with the NIA Fogarty-Magnuson Fellowship Program, might provide three- to six-month training opportunities in geriatric medicine abroad.

#### Clinical Base and Centers

Through the Gerontology Research Center at Baltimore, we have the advantage of collaborating with academic institutions such as Johns Hopkins, where we are discussing the possible development of further clinical programs. We are also investigating the possibilities at NIH, where some 100 of the 540 beds in the Clinical Center are unoccupied each day. The resources of the

Clinical Center provide a unique opportunity for studies in diagnosis, treatment and care of older people and for professional collaboration with other disease-categorical Institutes to examine particular problems.

The NIA also has plans to create a Clinical Center Consultative Service which could relate to the other disease-categorical institutes. In addition, we hope to attract medical students to the Clinical Center under clinical elective programs. They might later become part of the Baltimore research activitiy.

There is also a need for center development. We need the involvement and commitment of the private sector in this effort. we must be willing to support specialized single discipline centers ratner than simply allowing poor research to hide under the umbrella of a multi-disciplinary center.

we also need centers' that are time-limited, and they should be regional because, by being distributed around the country, they could provide practical core resources for scientists working in a variety of areas.

#### Miscellaneous Activities

A workshop was held to discuss the animal models so crucial to aging research. There were eight panels, ranging from farm animals to cats, ampnibia, salmon and non-human primates. On the basis of this information, we are examining the life tables, genetic makeup, and pathology of various species to determine their appropriateness for aging research.

A knowledge base is another resource we are seeking to develop. MEDLARS has not had an extensive vocabulary in terms of aging or gerontology. We are negotiating with the National Library of Medicine to develop a range of more effective connections in terms of aging.

Another important topic for consideration is human protection, especially in the case of older people who are potentially vulnerable to various types of studies. Last summer, we assembled a group of outstanding legal authorities, ethicists, and researchers in gerontology to think about the problem of informed consent and the very special problems that relate to older research subjects.

We also have a special concern for minorities--their access to research careers, the support of research affecting them, and the support of research by minority scientists regardless of what they are studying. There is an unfavorable differential life expectancy involving many racial and ethnic groups and, on the other hand, there are some very positive and interesting aspects of family and individual lifestyles, nutrition and physical fitness among these groups. We are working with the Division of Research Resources on minority biomedical support and we recently held a workshop with representatives of various minority groups.

An especially important area is information dissemination and transfer of knowledge. There are 7,500 voluntary hospitals

in the United States and more than 4,000 do not have libraries. Only 200 to 300 have teaching programs. We encounter a problem when we try to carry out our responsibility to transfer knowledge to the medical community when that community and its institutions are not prepared to receive that information or to respond to it.

Many of the so-called "diseases of civilization" may really relate to another important area, self-care--our own health practices, food, exercise and rest. Physicians, by and large, have been taught only to advise older people to "take it easy" and to prescribe drugs. There is not a very comprehensive well-thought-out prescription that relates to the totality of human health practice, including physical fitness and nutrition and only indirectly involving drugs. Consensus development seems to be as applicable to the entire problem of health practices as it is to specific disease entities.

Some 200 diseases account for about 80 percent of contacts with doctors. We will investigate the possibility of developing guidelines for these 200 basic disease entities in regard to older people.

We expect to develop an intramural NIA program presence in Bethesda to interact effectively with the other Institutes. By 1980, we hope it will be part of an ambulatory care research facility involved with the care of older people. Certainly, any ambulatory care facility would lend itself to studies in diagnosis, treatment and prevention.

To fully tap the intellectual resources of the Institute, we held a week-long planning meeting in the fall that brought together the extramural and intramural staff. Out of this intensive session came a sharper focus on areas for future exploration and emphasis. The process will be continued through a seven-member team for year-round review and evaluation. Once each year a similar concentrated planning period will be held for the total staff: intramural laboratory chiefs, program officers, and Extramural and Collaborative Research Program professional staff.

In the early spring, NIA will sponsor a major conference on nutrition. We don't yet know, for example, the nutritional requirements or the nutritional status of Americans over 65. We hope to work with the various Institutes in the area of nutrition, an important subject to many of us.

#### Constituency Attention

The elderly of the United States are not only increasing in numbers, but they are growing in vigor and militancy, as well as being more fully informed and organized. We consider it a positive result that in our first year of operation the NIA received more than 7,000 letters of inquiry, not counting casual communications. We were called upon for numerous public appearances to which Institute leadership responded. Increasingly close contact is being maintained with such organizations as the AARP/NRRIA, National Council of Senior Citizens, Grey Panthers,



National Association of Retired Federal Employees and the Former Members of Congress.

The Director recently received a friendly subpoena from the Civil Rights Commission to testify as to the problems of discrimination in delivery of services to older people. He testified, for example, about hospitals that have quotas on the admission of older people. In one state, only 10 percent of those who do not have an attending physician, even with Medicare, can be admitted to the hospital. The others are sent to a public hospital. Since only one out of every 10 people over 65 actually has an attending physician, the majority are rejected under the quota system or must make special arrangements.

The Director and Special Projects Officer serve on a research panel concerned with the handicapped, created by the Committee on Science and Technology and headed by Rep. Olin Teague. The panel brings together the various federal agencies that have an interest in prosthetics and technology transfer. Also included are representatives of consumer groups, the handicapped and disabled, and industry.

NIA ANNUAL REPORT  
JULY 1, 1976 THROUGH SEPTEMBER 30, 1977  
OFFICE OF THE DIRECTOR  
INFORMATION OFFICE

The legislation that created the National Institute on Aging specified that the Institute should carry out public information and education programs designed to disseminate as widely as possible the findings of Institute and other relevant aging research and studies, and other information about the process of aging which may assist elderly and near-elderly persons in dealing with all Americans in understanding the problems and processes associated with "growing old."

The Information Office was created to fulfill this mandate. During FY 1977 the Information Office has grown and has begun to take shape. Some of our long-range goals are as follows:

- To change the negative image of aging and the aged in the public mind.
- To provide the public, and especially the aged, with information on the special problems and needs of the old.
- To provide physicians and medical students with information which will help them improve the quality of care they give their older patients.
- To respond quickly and comprehensively to public inquiries about the NIA's mission and programs.
- To assist individuals in obtaining necessary information when the correct source is not the Institute.
- To demonstrate that research is both practical and cost-containing and that research can provide short-term benefits to which the general population can relate and from which they will benefit.

- To demonstrate to the public that new knowledge derived from research is the very foundation on which a high quality service delivery program rests.
- To coordinate with other federal agencies having programs in aging to avoid duplication of effort.
- To establish better working relationships with our grantees so that we may work together to transfer information more rapidly.
- To sensitize other NIH Institutes to the special problems and needs of the aged.
- To comply with the Freedom of Information Act.

During our first year the following was accomplished:

1. Response to public inquiries (telephone calls, letters, requests for publications): over a five-month period, from May to September 1977, the Information Office handled 3,000 inquiries, which equals approximately 7,200 requests per year. The GRC branch of the Information Office handled an additional 280 public inquiries and distributed an additional 4,000 publications, reprints, and reports since July 1977.
2. Press relations: we have held one science writer seminar to present the "state of the art" and hopefully interest the science press in aging; we have prepared and distributed 19 press releases and announcements; and arranged for 67 media appearances NIA staff, most notably the Director. The Institute was represented on all major networks and in such newspapers as the Washington Post, New York Times, Baltimore Sun, Baltimore News-American, and Indianapolis Star. National magazines such as Time, U.S. News and world Report, Harper's Bazaar, National

Geographic, Medical World News and Modern Medicine have also covered the Institute. Media contacts have been maintained via a special mailing list for media representatives.

3. Articles: we have prepared the following articles for publication in professional and lay journals:

"Early Directions for the NIA" in The Gerontologist

"The Need for Teaching Geriatric Medicine" in Geriatrics

Preface to a book on Immunology and Aging

"Caring Families" in the National Observer

"Trends in Training in Research Gerontology" in Educational Gerontology

"A Good Age" (book review) in Medical World News

"On The Other Hand: Geriatric Medicine" in Medical World News

"Psychosocial Aspects of Reproductive Aging" chapter in book on The Aging Reproductive System

"Funeral Societies," "Overuse of Tranquilizers," "Exercise, the Neglected Therapy," "What Everyone Should Know About Drugs: Long Overdue," "Why Shouldn't Providers and Consumers Join Together in an Alliance for Older People?" "Is There an Ideal Form of Care of the Old?" and "Nursing Home Care: An Impossible Situation Unless..." all for the International Journal of Aging and Human Development

"Research Programs of the NIA" in Public Health Reports

"Aging in America" in the Journal of Long Term Care

"Aging" chapter in book on Psychiatry in General Medical Practice

Geriatric Medicine: The Necessities" in Connecticut Journal of Medicine

"Ageism and the Humanities" for tentative publication in  
The Gerontologist

"Aging" for the Encyclopaedia Britannica 1978 Medical and Health Annual

"NIA Support for Research on Aging" for newsletter

"Aging: A Challenge to Medicine" for use in newsletters of  
local medical societies

"Geriatric Medicine: The Imperatives" in the New York Journal of Medicine

"Aging and the Elderly" epilogue to a book by the same name

"What You Should Know About Drugs" in Pharmacy Times

"One in Ten," "Our Future Services," "Old Age" all in  
NIH: The Search for Health

"Mission of the NIA" in Journal of the American Geriatrics Society

"Aging and Mental Health" chapter in book Clinical Geriatrics

"Too Old, Too Sick, Too Bad" book review in the Washington Post

"What To Do With the Old Folks" book chapter for the Ann Landers  
Encyclopedia

"Practical Priorities for Research on Aging" in Retirement Living

"Welcome to the Middle Years" book review in the Washington Post

"Aging and the Aged" for Funk and Wagnall's Encyclopedia  
News articles for the NIH Record

4. Brochures and flyers: we have written, supervised the layout, printed, and distributed the following brochures and flyers:

Our Future Selves

NIA brochure (interim; final is in press)

Research Programs of the NIA (reprint of article)

Special Research Awards

Special Report on Aging

Medicine and Aging

NIA basic packet holder

Saints and Sinners: The Baltimore Longitudinal Study of Aging

Background Statement: GRC

Science Writer Seminar Series (7 pamphlets; in press)

Welcome to the NIA (in press)

5. Conference summaries: we have prepared summaries of NIA-sponsored conferences on geriatric medicine and economics, as well as editing National Advisory Council on Aging transcripts and the Director's remarks at conferences on the protection of human research subjects, epidemiology and aging, and senile dementia.
6. Communication with constituent organizations: we have attempted this through various mailings, telephone contacts, and numerous public appearances by the Director.

Although we are quite pleased with what has been accomplished in our first year with such a limited staff, we are keenly aware of our failures in certain areas. We expect that with an ambitious

program for the future we will make up some of our deficits in the actual transmittal of information which will be of immediate use to the public and to our constituents.





## NIA ANNUAL REPORT

### Report of Extramural and Collaborative Research Program

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OCTOBER 1, 1976 THROUGH SEPTEMBER 30, 1977  
REPORT OF ASSOCIATE DIRECTOR FOR EXTRAMURAL AND  
COLLABORATIVE RESEARCH PROGRAM

The reporting period was a crucial, transitional time for the Extramural and Collaborative Research Program (ECRP), just as it was for the entire Institute.

The first Associate Director for the Program, Dr. Betty H. Pickett, was recruited and selected in the last half of calendar year 1976; unfortunately, for reasons quite outside the control of NIA, her appointment was delayed until June of 1977. However, owing to the generosity of the National Institute of Mental Health, from which Dr. Pickett was recruited, a "detail" assignment to NIA could be arranged which in some measure effectively obviated what had promised to become a deleterious and lengthy delay.

The Program, which supersedes the former Adult Development and Aging Branch of the parent Institute (NICHHD), was given its present designation at the time of appointment of the Associate Director. In May of 1977, the ECRP, along with the Grants and Contract Management unit, all of whom had been housed in the Landow Building, moved into Building 31 on the NIH campus, into quarters contiguous with those of the Director and his staff. The net effect of the move has been extremely salutary, in that opportunity has become available for development of close, working relationships among the key elements of the Bethesda staff of the NIA. Daily, the beneficial effects of this shift become more apparent.

Despite the administrative and geographical stresses and strains, the reporting period has been active, with the promise of significant movement in several significant areas, including pharmacology, nutrition, behavioral and social sciences, minority initiatives, and others to be described below. The basic biological areas, always strong, have maintained and in some areas accelerated their momentum.

The factors which could hold back further development of the Program are the usual ones - physical, human and fiscal resources. There is some reason for guarded optimism in regard to each of these needs and it is hoped that Fiscal Year 1978 will witness improvements in each area. Personnel needs for ECRP have been subjected to thoughtful assessment. Plans are in place for modest additional staff acquisitions by the end of Fiscal Year 1977 (one professional and two clerical positions will be added to the present complement of six professionals and seven support staff). It is hoped that Fiscal Year 1978 will provide further modest, additional staffing along with space for that added complement.

A very critical problem in program organization and development is analytic capability. Generically, of course, it is essential to have the capacity to take stock, analyze trends and directions; in a new program or one which, like ECRP, has a history, but is heading into an altered and expanded future, analytic capacity is crucial. To date, although NIA has not developed much function in this area, initial steps have been taken. Development of even the most simple basic data such as those to be reported below is, at present, cumbersome. It is hoped that Fiscal Year 1978 will bring significant improvement in NIA's reporting and analyzing abilities, and that the next Annual Report will feature a more sophisticated, analytic approach.

Since May of 1977, the Associate Director and professional staff of ECRP, in consultation with the Director, NIA, have expended considerable effort toward a reorganization of ECRP into substantive program areas. The process is close to completion, but not ready for public dissemination at this date; it is clear, however, that the eventual programmatic breakdown will be highly substantive, and aligned appropriately with NIA's public statements.

#### PROGRAM ANALYSIS TABLES

To date, \$21,687,000 millions were expended for 276 extramural and collaborative research awards within the reporting period.

The Institute's program "activities" are those commonly in use by NIH Institutes. For the total reporting period, distribution of grants and dollars across "Activities" (for all types of grants and contracts, including new, renewals, continuation/ and supplemental awards) can be found on Table I attached.

The right-hand column of Table I indicates continuation of the fund distribution shift described in NIA's 1976 Annual Report, e.g., for the total reporting period 42% of ECRP research grant funds (across all research program mechanisms) were devoted to awards for large programs (program projects and the single core-center grant), while 54% of ECRP funds were provided for individual, investigator-focussed awards, such as the Special Research Awards, Regular Research Project Grants, and Research Career Development Awards. Within the Institute's small research training program, most of the funds were expended on institutional awards (T-32's), as opposed to Individual Post-doctoral Research Fellowships.

The distribution of funds by "type" of award, i.e. among non-competing renewal awards (or prior commitments) and the varieties of competing awards (new grants, competing renewals and competing supplemental awards), is displayed in Table III attached, for all program "activities." Forty-eight percent of ECRP funds were expended on non-competing continuations, only about 6% on competing renewals, some 38% of new awards, with about 5% for supplemental

grants. This, too, indicates a shift from the trend reported in the 1976 Report which had indicated a minimal amount available for new awards.

#### OUTLOOK FOR FISCAL YEAR 1978

Program allocations within NIA for FY 1978 have, of course, not been made at the time of preparation of this report. The appropriation bill for NIH has not yet been passed and signed by the President. But based on the House-Senate Conference, we can look forward to some modest increases in funds available for the Institute's Extramural and Collaborative Research Program. With an anticipated more than 20% increase in the overall funding for the Institute (from \$30 to \$37 million dollars) we can anticipate commensurate enhancement of our ability to support investigative research on aging. Although ECRP's specific allocation is unknown at present, we hope that it will allow some reduction of the annual load of unfunded approvals. However, with the anticipated increase in numbers of applications submitted in response to program development initiatives and the increased costs of research, it may not be possible to reduce the level of unfunded approved applications significantly below this year's expected level of \$4.5 million.

#### SUBSTANTIVE ANALYSIS OF EXTRAMURAL AND COLLABORATIVE RESEARCH PROGRAM AWARDS

In the absence of a developed program analysis capability, only the crudest, substantive program statistics can be provided. It is possible, nevertheless, to determine the coarse parameters of ECRP's research and research training program emphasis from the following disciplinary breakdown of FY 1977 data only (10/1/76-9/30/77).

Table IV (attached) shows that within research "activities," 59% of the funds can be categorized as in support of biological research, 25% for behavioral and social sciences, 7% for clinical research, and the rest for studies categorizable under more than one rubric. Research training awards are more difficult to categorize neatly, with more than 40% of the funds going to awards which are multi-categorical in nature. Research contracts appear to be mainly biological in nature.

A thoughtful assessment of the NIA's research training program is underway at the date of this report. Issues under study are how to maximize the training grant authority to provide the necessary research manpower for a sharply increased research effort on aging. At present, 140 trainees are supported under the NRSA's Institutional Research Training Grants, 29 Fellows under the NRSA's Individual Postdoctoral Research Fellowship. By discipline, 61 of these researchers-in-training fall within the biological areas, 7 within clinical research, 50 and 51 respectively within the behavioral and social sciences areas. About 55% of the total group of trainees are in the

predoctoral phase of their education; the remainder are postdoctoral. By far, the preponderance of predoctoral trainees during the reporting period have been in the behavioral and social sciences areas.

The Institute's Congressionally mandated responsibilities indicate the need for an expanded effort in the behavioral and social sciences. Only limited expansion has resulted from the modest programming efforts mounted so far. For the future, intensified developmental activities will highlight psychological and social research issues through Conference and Program Development Meetings, through Requests for Applications and through staff visits to research institutions, publicizing of NIA's research goals and guidelines. One of the key factors in furthering research on behavioral and social science problems is the recruitment of additional accomplished research investigators from these disciplines into aging. Some additional manpower will be to research and will be recruited under the modest funding for research training available to us. But most, necessarily, will have to be skilled behavioral and social sciences researchers not now working on problems of aging. At least two of the program development meetings to be held during the upcoming year will attempt to do just kind of recruitment. (See the subsequent section on Program Development Meetings for details.)

#### REVIEW OF NIA'S APPLICATIONS

Additional Study Section capacity has been added for the review of NIA's research grant applications, focussed on the social and behavioral sciences areas; in the latter part of FY 1977 several reviewers skilled in social science aspects of aging research were added to an existing DRG review group (Population Study Section). Nevertheless, the Institute continues to be extremely concerned about the quality and quantity of aging research projects especially in the behavioral and social sciences, which undergo peer review, both within DRG and within the Institute's own Aging Review Committee. As before, all Program Project and Institutional Research Training grant applications are reviewed by the Aging Review Committee.

Figure 1 and Table IV (attached) summarize recent workload trends both in terms of applications reviewed by the National Advisory Council on Aging (NACA), and their approval rates. For submissions to the past three meetings of the NACA, the trend is clearly upward; this can be expected to continue, as program development efforts accelerate in the period ahead.

Approval rates for all aging applications are not out-of-line with approval rates across the board for NIH.

Review of NIA's applications with the exception, as noted above, of those handled by the Aging Review Committee (NIA's only internal Initial Review Group) is carried out by some 30 DRG Study Sections (with an occasional review by an ADAMHA Initial Review Group).

NIA's leadership is launching efforts to strengthen the Institute's relationships to the Study Sections which review its applications; a series of planned visits to Study Sections by the Institute's Director and Associate Director for Extramural and Collaborative Research Program will supplement the regular attendance at appropriate Study Section meetings by respective ECRP staff.

#### PROGRAM DEVELOPMENT EFFORTS

1. New Referral Guidelines for NIA were drafted in FY 1977. At the time of this report, negotiations continue with the several respective Institutes with which NIA has mutual or overlapping interests on a variety of problems (such as senile dementia, normative behavioral aspects of aging, nervous system aging, etc.) When these negotiations are completed, the Guidelines, or some condensed version thereof, will be published in the NIH Guide. In brief, the program areas covered in the Guidelines are as follows:

Biology of Aging, including fundamental studies of the biological processes of aging, utilizing biological, biophysical, physiological, biochemical approaches, and involving all levels of biological function; cellular aging, organ change with aging, changes in immunological function with aging, metabolic alteration with aging are all within NIA's interests. Studies in vivo, in vitro, on both humans and experimental organisms are supported.

Medical and Clinical Aspects of Aging, including epidemiology and demography of disease in the aging and aged, geriatric medicine, the factors associated with prevention of illness and infirmity; health maintenance and promotion of health in the aging and the aged, e.g., nutrition, exercise, environmental and socioeconomic variables, special pharmacological problems affecting the aging and the aged, such as drug-drug interaction, over-prescription, overdosage, problems of changes in the various bodily systems with age, e.g., nervous system, reproductive system, musculoskeletal, connective tissue changes associated with age, need for special devices and techniques to increase the mobility and self-care capacity of the frail and handicapped elderly.

Psychological and Behavioral Aspects of Aging, including studies of cognitive, psychomotor, perceptual, sensory social, personality and attitudinal changes associated with the aging process; psychobiological and neuropsychological aspects of aging. Studies of aging in both humans and animals can be supported in this area as well as in the biological sciences.

Social and Societal Aspects of Aging, including adult socialization into the aging process, attitudinal changes as accompaniments of aging, family and intergenerational patterns associated with aging, demography and epidemiology of aging, socioeconomic factors in morbidity and mortality differentials

between the two sexes and among the different subcultural and ethnic segments of the aging population; changing age-structure of the population and its impact on economic and societal function; retirement decision-making, and effects of retirement at different ages on individuals and the society; functional capacity of aging individuals for work; effects of social and institutional factors on health and well-being of the aging and the elderly, such as housing, living arrangements and similar factors.

2. Special Research Award Guidelines were issued early in the reporting period, and modified later. The final, modified version of the Special Research Award, issued in the NIH Guide, August 10, 1977, bring it more or less into line with similar programs in several other NIH Institutes, providing for up to 3 year's support, up to \$90,000 total funding for the period, and allowing for such items as Personnel, Equipment, Supplies, Travel, Other Expenses and Indirect Costs, (including a limited amount for the principal investigator's salary).

### 3. Requests for Application (RFA)

Pharmacology and Aging -- A program announcement concerning NIA's interest in receiving research grant applications of problems relating to drugs and aging appeared in the NIH Guide, March 1, 1977. Since that date, only a few responsive research grant applications have been received; of these none has been reviewed to date. An unusual, cooperative inter-Institute arrangement with the National Institute of General Medical Sciences provided the "personpower" for the interactions with investigators responding to the RFA, as well as for the planning and organization of a Pharmacology/Aging research Workshop scheduled for September 15-16, 1977. See subsequent sections for further discussion of this area.

Nutrition and Aging -- In response to the growing concern both within NIA and NIH and across the Federal Government for nutrition as an overarching factor in aging and disease, an announcement of NIA's interest in receiving relevant research grant applications on a broad array of problems within this area appeared in the NIH Guide, August 10, 1977. At the time of preparation of this report, of course, no responsive applications have yet been received. A full report of the response to this initiative will be provided in the Annual Report for FY 1978. Additional information on NIA initiatives in Nutrition is contained in a subsequent section of this report.

Joint NIA/NICHD Nutrition Effort -- In response to a request from the Director of NIH, NIA and NICHD, NIH's two longitudinal Institutes, have joined forces in what has developed into a continuing effort concerning research on "clinical nutrition." Personnel for the initial phase of this effort was drawn from both Extramural and Intramural staff.



4. Minority Initiatives -- During the reporting period the Institute made explicit its goals of increasing research activity specifically focussed on problems relevant to minority aging and of increasing the minority research manpower involved in research on aging. Agreements have been entered into between the Institute's ECRP and the Minority Biomedical Support Program of the Division of Research Resources as well as the Minority Access to Research Careers Program of the National Institute of General Medical Sciences. To date, a few awards, totalling \$177,816 have been made under the former agreement, none as yet under the latter. ECRP staff includes two minority scientists, who devote not insignificant portions of their time to furthering the Institute's stated minority goals. As described below, Program Development Meetings furthering these goals were among those held during the reporting period. ECRP staff, along with NIA's Director, participated in NIH-wide efforts to stimulate additional minority involvement in biomedical and behavioral/social research on aging.

5. Program Development Meetings -- As indicated in the preceding sections, a variety of staff efforts have been made during the reporting period to further stimulate aging research. Some involve obtaining scientific advice on program directions and assessments of the state-of-the-art, in one or another fields, subfields or concerning particular problems or techniques in aging research. Some of the activities involve partial support for scientific meetings organized outside the Institute which ECRP staff judge will aid program development efforts. Other meetings, some very informal consultations, other more formal events, resulted more directly from staff initiatives. Some of the meetings will result in published reports of proceedings; others will result in less formal reports. All of these activities stem from the Institute's urgent need to attract the attention of additional investigators to needs of aging research, to obtain skilled advice in setting Institute research support priorities, to identify specific areas in which further support is needed to move the field ahead, and to stimulate the submission of additional, high quality proposals for research and research training support.

Program Development Meetings during the reporting period in which ECRP staff were focal include the following:

Program Development Meetings

ECRP Staff Leadership

Immunopathology

Lester Smith, Ph.D.

Various immunopathologies of man can be found among animal models. We are interested in obtaining a data base on those pathologies which may have their etiology in a defective immune system. This workshop concerned state-of-the-art knowledge of major pathological changes which involve vital organs of the immune system in aging rodents.

Workshop on the Immune System of the Aging Rat    Lester Smith, Ph.D.

The knowledge gained from this workshop will allow the new program efforts around the rat as a model to study immunology and aging. The rat model should be further studied for several reasons, chiefly among which are the opportunity to do repeated sampling, the quantity of pure lymphocytes which can be obtained, the known T & B cell composition of macrophages and the absence of macro.

Symposium on Health and Black Aged

Shirley Bagley, M.S.

Supported part of the Annual Meeting of the National Center on Black Aged. Status of research and new areas where research is needed were foci of discussion.

Research on Minority Elderly

Shirley Bagley, M.S.

Small workshop to provide advice to NIA on the development of a research program on psychological and social factors concerning aging in minority groups.

Program Development Meetings

ECRP Staff Leadership

Towards an Anthropology of Aging

Shirley Bagley, M.S.

Focussed on cross-cultural research on aging, including questions that need to be answered by research, criteria for site selection; will result in a publication.

Summer Workshop for Behavioral & Social Scientists

Shirley Bagley, M.S.

Aimed primarily at investigators new to the field of aging, to help in development of research ideas suitable for submission as grant applications in aging.

Planning Workshop for a Task Force on "Relevant and Appropriate Vertebrate Species for the Study of Aging" Don Gibson, D.V.M.

A Workshop Conference was convened in mid-December 1976 to preliminarily review and evaluate the relevance and appropriateness of selected vertebrate species including mice, rats, dogs, cats, non-human primates -- mammals, amphibians, birds and fishes for research on aging. The Workshop formed the basis for planning the development of a task force to assess selected vertebrate species. Based on the advice and recommendation of the Workshop, a contract was developed with the National Academy of Sciences, National Research Council, Institute of Laboratory Animal Resource, to assemble and conduct a task force evaluation of selected vertebrate species for the study of aging.

## Program Development Meetings

## ECRP Staff Leadership

### Exploratory Workshop on Development of an Organs, Tissues, and Fluid Bank from Aged Animals

Don Gibson, D.V.M.

The purpose of the Workshop is to identify specific materials needed by investigators in aging, its preparation, storage and distribution. Also, to facilitate the exchange of rare or unique material from less commonly used aged animals as their tissues occasionally become available.

### Social Science Consultation

Betty H. Pickett, Ph.D.  
(with Robert N. Butler, M.D.)

Leading social scientists met with NIA leadership to exchange ideas on social science research needs in aging, strategy for research program and research manpower development in social science/aging interface.

### Pharmacology and Aging

Betty H. Pickett, Ph.D.  
Sara Gardner, Ph.D. and  
NICMS staff  
Robert N. Butler, M.D.

The purpose was to review the current status of pharmacology and aging, bring together scientists actively working in various relevant areas, to discuss problems which may be impeding progress and point out needed new directions for research efforts.

### International Workshop on Cell Tissue and Organ Cultures in Neurobiology

Donald G. Murphy, Ph.D.

Conferees examined nervous tissue culture in the light of new developments in concepts and technologies in this field. A review of the present state of knowledge was provided as a basis for the use of nerve tissue culture in contributing to the solving of neurobiological problems. The Workshop provided a forum for exchange of ideas among participants and an edited book is

Program Development Meetings

ECRP Staff Leadership

in preparation from the Workshop.  
(Federoff, editor)

Mycoplasma Infection of Cell Cultures

Donald G. Murphy, Ph.D.

In-depth sessions were conducted of basic properties of the mycoplasmas, the isolation and detection of mycoplasmas in cell culture, biochemical and microscopic approaches to detection of mycoplasmas in cell culture, and the effects of mycoplasma contamination on cell culture. The Workshop also covered prevention, control, and elimination of mycoplasma contamination of cell cultures. Precedings of the Workshop are to be published: McGarrity, Murphy, and Nichols, editors.

Gordon Research Conference on the Biology of Aging

Donald G. Murphy, Ph.D.

Enabled presentation and exchange of ideas on a wide range of research within the field of biomedical aging. Consistent with Gordon Research Conference policy, the meetings were relatively informal and no publication of reported data will be made.

Caenorhabditis elegans Workshop

Donald G. Murphy, Ph.D.

Participants exchanged data and discussed concepts on all aspects of research with this organism, the laboratory nematode. The uses of C. elegans as an aging model was pursued in the context of genetics developmental biology, nutrition, and theory. Studies were reported in which aging mutants are being sought.

Program Development Meetings

Cellular Senescence and Somatic Cell  
Genetics: DNA Repair and Cellular  
Senescence

Somatic cell mutations from the basis of widely recognized theory of aging. DNA repair system, their capacity and accuracy play a key role in counteracting the induction of mutations. This workshop provides comprehensive coverage of the state-of-the-art of the field of DNA repair, the special attention given to problems of aging research. (To be published: Nichols, W.W., and D.G. Murphy (eds.) DNA Repair Processes and Cellular Senescence. Symposium Specialists, Miami, Florida (in press).

ECRP Staff Leadership

Donald G. Murphy, Ph.D.

New Approaches to Aging Research via  
Cell Culture

Donald G. Murphy, Ph.D.

Participants considered several candidate cell culture systems in the context of their potential for aging research. Of these the most promising were vascular endothelial cells and keratinocytes.

The Program Development Meetings will continue with the next Fiscal Year.

Within this program area the NIA plans, implements and supports research in cell biology, theoretical and evolutionary biology and investigations utilizing cell, tissue or organ culture, and invertebrates or plant model systems.

Cell Culture Studies of Aging:

Aging of human cells in culture has provided a major study focus for the gerontological research community. The phenomenon of decline in proliferative capacity of normal cell culture populations, leading to cell population death constitutes one of the extant gerontology fields most aggressively pursued. Progress in understanding this loss of function will contribute directly to hypotheses and experimental design for the more sophisticated use of cell culture systems as direct probes of human cellular aging. It will also provide the aging-knowledge contribution to the use of the same original cell line, but in the transformed state, as a model in cancer research.

The development of the field of cultured-cell gerontology was encouraged through NIH programmatic activities, the most visible of these being cell production and distribution facilities. The earliest of these contracted facilities was for the production and distribution of the human diploid cell strain WI-38, and as a consequence this became the primary line studied within the aging research community. WI-38 continued to be the major line studied for aging following termination of the contract in 1976. Subsequently, the original WI-38 stock ampules were inventoried by NIH and found to be in short supply, and thus reserved for vaccine production. During this past year the cell line of IMR-90, to replace WI-38 for research purposes, was introduced into the gerontological community specifically, and the scientific community in general, by the NIA. This standard line was developed and characterized through the NIA contracted cell bank at the Institute for Medical Research, (IMR), Camden, New Jersey. Additional standard lines are under development and should be available for release in the near future.

The techniques of the emerging field of somatic cell genetics are most promising for investigation of cell-culture senescence phenomena. For this and related purposes genetically marked cell lines are produced, banked and distributed by the NIA contracted facility at IMR.

Findings on NIA supported investigations on senescence of cells in culture reported in FY1977 include:

1. the observation of the presence of certain specific hormone binding sites on cultured cells and the suggestion that the presence of these functional reception sites is correlated with the proliferative state of the cell culture population,
2. that neither oxygen toxicity nor free radical reactions play a significant role in limiting the life span of normal cultured human cells under ambient oxygen tensions,

3. that in early population life span of cultured cells there is a appearance and subsequent loss of polyploid cells indicating a selection process operating in young cultures,
4. the development of a sensitive and reproductable methodology measuring x-linked and related autosomal-linked enzymes, for the evaluation of the biological age of a cell population.

Areas being encouraged for development include cell lineage studies as a means to gain insight to aging phenomena at the tissue and population cell level, and research on differentiated cell types. The problem of aging of cell populations in culture will continue to be pursued aggressively.

Services to be developed in support of the program include a mycoplasma contamination testing facility (a contract is to be awarded early in FY 1978) and the possibility of a cell line identification service will be taken into consideration.

#### Invertebrate Model Systems:

The problems of understanding mechanisms of aging are of such a magnitude that systems for study less complex than man and other mammals are highly desirable. Because the fundamental mechanisms of aging are not understood in any organism which senesces, short of catastrophic death (loss of feeding apparatus in some invertebrates, for example), even comparative studies of aging become highly desirable on simple organisms. The genetic, molecular, and cellular apparatus of most lower organisms are remarkably similar to those of man, thus even differing aging mechanisms in widely dissimilar organisms, if discovered, are likely to contribute to testable hypotheses in higher organisms and man.

This program currently supports research in invertebrates ranging from protozoa to arthropods. Special emphasis has been directed to the use of the laboratory nematode, Caenorhabditis elegans, as a model for aging research. This organism offers advantages of relatively simple morphology, short-life span, and a rapidly evolving base of knowledge of its genetics. It can be studied on an individual animal basis, and also produced in large quantities for biochemical studies.

Recent studies demonstrate that:

1. that this nematode's life span can be altered during any part of the life cycle by change in temperature or food concentration,
2. it accumulates fluorescent pigment resembling lipofuscin as it ages,
3. that it becomes less sensitive to ultraviolet radiation with age,



4. parental age and parental life span have relatively small effect on progeny life span,
5. the duration of the "dauer" (an active, but non-feeding facultative state) has no effect either on the post-dauer life span, egg production or egg visibility. Nematodes that have been in the "dauer" state for 60 days (normal life span without dauer is less than 30 days) have the same post-dauer life span as nematodes that were in "dauer" only five days.

These and other studies provide the basis for more detailed genetic and biochemical analysis of life span and senescence in this nematode.

The laboratory nematode will continue to receive program emphasis. In addition to this, a small organism, *Volvox*, will be encouraged as an aging model. Data will be developed for expanded program effort, in future years, on *Drosophila* and the ciliated protozoa.

Program Publications:

Nichols, W.W., D.G. Murphy, V.J. Cristofalo, L.H. Toji, and S.A. Dwight, 1976. Characteristics of a new human diploid cell line, IMR-90. Science 196(4285): 60-63.

Nichols, W.W., D.G. Murphy, Regulation of Cell Proliferation and Differentiation, Vol. 1, Plenum Press, New York, 1977.

Genetics and Comparative Aging  
Collaborative Research, ECRP

Contract Number: AG-2-2755

Contract Title: Quantitative Studies of Aging Human Diploid Fibroblasts  
in vitro

Contractor: University of Vermont  
(Principal Investigator: Dr. Marlene Absher)

Money Allocated: Terminated February, 1977

Objectives: The Contractor described the division patterns and cellular lineages of human cells grown in culture utilizing time-lapse cinematographic, autoradiographic and computer analysis and model simulation techniques.

Publications resulting from this contract:  
Principal Investigator: Dr. Marlene Absher

Absher, P. M., Absher, R. G., and Barnes, W. D.: Genealogies of clones of diploid fibroblasts. Cinemicrophotographic observations of cell division patterns in relation to population age. Exp. Cell Res. 88 :95, 1974.

Absher, P. M., Absher, R. G., and Barnes, W. E.: Time lapse cinemicrophotographic studies of cell division patterns of human diploid fibroblasts (WI-38) during the in vitro lifespan. In "Cell Impairment in Aging and Development". Eds. Vincent J. Cristofalo and Emma Holechova. Plenum Press, New York, N.Y., p.91, 1975.

Absher, R. G., and Absher, M. P.: A mathematical model for clonal growth of human diploid fibroblasts in vitro. Tissue Culture Association Meeting, June 1975, Montreal, Quebec. (abstracts)

Absher, P. M., and J. Absher, R. G.: Clonal variation and aging of diploid fibroblasts. Cinematographic studies of cell pedigrees. Exp. Cell Res. (In press)

Contract Officer:

Donald G. Murphy, Ph.D.

Murphy, D. G.: NIH Gerontology Research Support: Policy Evaluation of in Vitro Lineage Studies. Mechanisms of Ageing and Development, 4 (1975) 317-323.

Lineage studies in vitro and in vivo continue to be necessary for program balance. Consistant with the review conducted on this matter (see publication above) lineage studies will be encouraged through the grant mechanism.

Significance for Aging Research:

The human diploid cell in culture is a widely-studied model for aging. Populations of these cells double actively under standard cell culture procedures for many months, but eventually age and die. Although extensive research is being conducted on populations of such cells, the studies are being pursued without definitive knowledge of the division characteristics of individual cells and their progeny. There is evidence that "old" populations contain many "young" behaving cells, just "young" populations contain "old" behaving cells no longer capable of division. Cell lineage data were produced by Dr. Absher to the end of refining experimental design, cellular aging research concepts and hypotheses, and developing a capacity to relate cell culture studies of aging to the aging process in man.

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Genetics and Comparative Aging  
Collaborative Research, ECRP

Contract Number: N01-AG-4-2865

Contract Title: Selection, Production, Characterization and Distribution of Genetically Marked Cells for Aging Research

Contractor: Institute for Medical Research, Camden, New Jersey  
(Principal Investigator): Dr. Warren W. Nichols

Money Allocated: \$207,416

Objectives:

1. Provide standard, highly-characterized, extensively banked lines of normal human diploid fibroblast-like cells.
2. Provide banked and characterized, genetically marked or mutant cells identified as possessing features of value in probing mechanisms of cellular aging.
3. Provide standard, characterized, moderately banked lines of tissue-specific cell types.
4. Contribute to the development of the field of cellular aging theory, concepts, and techniques, through consultation and workshops, particularly in the areas of somatic cell genetics and cytogenetics.
5. Limited cytogenetic screening for aging research.

Significance to Aging Research:

The in vitro expression of genetic uniqueness of different cell strains offers powerful means to investigate mechanisms of aging at the cellular and sub-cellular level. This contract is to encourage the use of somatic cell genetics in studies of cellular aging; provide characterized, contaminant-free cell cultures to qualified investigators; and enhance research through communitative activities such as the annual workshop. The Institute for Medical Research resource is to be a focal point of grant program activities in cellular aging.

Since its inception, this cell repository has received 159 biopsies or cell lines. One hundred-forty-five of these have been successfully cultured and are frozen away. During the past year, 81 cultures of genetically marked, or mutant cells and 117 of the standard line IMR-90 have been shipped to investigators for the purposes of aging research (persons requesting the lines for research other than aging are charged a fee which is used to reimburse the NIA contract to IMR). A follow-up study is in progress to assess the eventual use of these cell lines and resulting research publications. This study is proving difficult and expensive.

"Fail-safe" storage, through alternate storage sites, for the standard cell lines IMR-90 and IMR-91) is being developed. The American Type Culture Collection, Rockville, Maryland, now holds a supply of these lines, and the W. Alton Jones Cell Science Center, Lake Placid, New York will receive a similar supply for long-term storage. A third alternate storage site is being sought.

The contractor has done preliminary studies on the use of isoenzyme polymorphism to identify, or "finger-print" cell lines as a means to ascertain accuracy of cell line designation and recognize cross-contamination. This has been carried out on 31 cell strains and compared to 9 standards. Seventeen strains can be detected by isoenzyme patterns.

Characterization and banking of the standard lines should be completed early in FY78. Future cell line acquisition emphasize differentiated, or tissue specific, cell lines of demonstrated or potential usefulness to aging research.

## EXPERIMENTAL ANIMAL MODELS FOR THE STUDY OF AGING PROCESSES

Hypothesis about aging processes usually cannot be tested directly in the human. Therefore, the research programs of the National Institute on Aging (NIA) are heavily dependent on animal models for the study of aging processes. Because of the increasing dependency on the development of animal models for basic and applied studies of aging processes, the selection, development, characterization and availability of aged defined animals is of critical importance to progressive advancement of research programs on aging. In view of this critical need, NIA has taken the initiative to support, develop, acquire, evaluate and provide aged animals and the information necessary for the assessment of selected strains and species of rodents and other vertebrates for the study of aging processes.

A major contribution of the Extramural and Collaborative Research Program to research on aging is the development of colonies of characterized, pathogen free aged mice and rats for use in research projects supported by NIA, and pilot studies that may suggest formal studies on aging. This program over the past seven years has supported collaborative research projects to establish:

1. the feasibility of developing and providing pathogen free aged rats and mice from a central breeding laboratory.
2. the criteria necessary to rear aged animals independent of infectious disease,
3. that aged animals free of pathogens can be shipped by commercial carrier to laboratories throughout the USA,
4. the age specific characteristic of selected strains of aged, inbred rats and mice including pathologic and age associated morphologic and functional changes,
5. actuarial data basic to the planning of studies in aging animals,
6. a program of allocation and distribution of aged mouse and rat strains from a central resource colony,
7. a reporting of data and information on the strain developed for research on aging.

Late in 1974, the Extramural program on aging began a modest program to provide aged mice and rats to investigators for research on aging, since the earlier studies had clearly demonstrated that pathogen free animals

survived well beyond a 24 month mean survival time. Initially, this program was limited to investigators supported solely by research grants from the Aging Program and in close proximity to the central breeding laboratory.

Currently, the National Institute on Aging maintains under commercial contract, a colony of Fischer 344 rats (male and female) and C57BL/6 (male and female), BALB/c (male), and CBF/1 (male), (a hybrid cross of BALB/c X C57BL/6) inbred mice. Aged rats and mice, 3 to 30 months of age are provided to investigators for pilot studies in anticipation of later submission of a research grant application, as well as for predoctoral candidates whose studies will form the basis for a dissertation on aging research. During the past three years, this program has provided 6,065 aged mice and 8,523 aged rats in support of over 100 projects on aging research. This includes support of extramural and intramural projects, research grants, contract and pilot studies as well as the availability of biological materials to ancillary projects.

In addition, this program has provided the first comprehensive characterization data that establishes a basis for selection between the strains and species of animals. This includes over 15 scientific publications on the use of the strains maintained by the National Institute on Aging.

#### NUTRITION AND AGING

Research on nutrition has become an increasingly important component of the research programs of the National Institute on Aging. This program will be broadly aimed at establishing the interrelationship between dietary intake, disease prevention and optimal health maintenance in the aged.

The adequacy of the level and balance of intake of essential nutrients provided by the diet affects the functional efficiency and morphologic structure of all organisms and probably the psychological responsiveness and social activity of the aged. Throughout life, the health status and response of the individual to the challenges of disease, environmental stress and aging depends upon the quality and quantity of nutrients consumed as well as the adequacy of current dietary intake. Thus, research in appropriate animal models and human population or nutritionally mediated influence on cellular morphology and function, endocrine response, metabolic processes, immune function, and may provide some of the most significant answers needed to moderate or prevent the development of mental and physical disabilities that currently characterize progressive deterioration that occurs in body and organ function with age.

It is clear that because nutrition influences all of life's processes, it is of primary importance in aging research on prevention of the major physical and mental disabilities that occur with advancing age. For example, a host of pathophysiological processes are linked with excessive or deficient dietary

intake of essential nutrients, vitamins and trace minerals. These include diabetes, obesity, hypertension, osteoporosis, immune function, and mental acuity.

Although the present research base on nutrition in aging is modest and primarily limited to a few studies on nutrition and health status, and the influence of dietary intake on physiologic and pathophysiologic response, the National Institute on Aging is planning the development and programming the expansion of its research base on nutrition and aging. This program is already under way. As noted above, a request outlining the important areas of research on nutrition and aging has been developed and circulated to the scientific community. Emphasis of this request is on encouraging the submission of research grant proposals to study the effects of nutrition on the health and well being of the aged as well as basic aging processes. The priority areas identified by NIA in which studies are encouraged include:

1. Nutritional status and requirements of the aged, particularly the influence of therapeutic modalities on nutritional status, i.e., amino acids, protein, vitamins, minerals, etc.
2. The effect of social, psychological, and economic factors on dietary intake.
3. The effects of dietary modification on health, longevity, pathologic processes and physiologic responses, particularly immune and endocrine response.
4. Nutrition, cellular structure and function as a function of age.
5. Identification and characterization of specific models for nutrition and aging research.

The development of these research areas in nutrition and aging will be coordinated and developed in cooperation with other Institutes to insure a maximum impact of the research findings on health of all segments of the population.

#### ENDOCRINOLOGY AND AGING

The effects of aging on the endocrine system is varied, being marked in some glands and slight or undetectable in others. Of the glands of the endocrine system, the most dramatic changes that occur with increasing age are those related to the sex glands leading to the menopause in women and a decrease in the secretion of testosterone in men. It is apparent in other glands such as the thyroid and adrenal that there is a gradual decrease in



function with age. The influence these decreases have may be related to some of the deteriorative changes in mental function and in strength that occur with aging.

It is clear that modification and imbalances in endocrine function are dominant characteristics of aging processes in humans and animals, affecting one sex as well as the other. Furthermore, these modifications are major determinants of the function of many, if not most, hormonal target tissues and organs. The tissue and organ changes in morphology and function include hyperplasia of the uterine endometrium, hypertrophy of the prostate gland, ovarian involution and cessation of estrogen production as well as changes in pancreatic function influencing the onset of adult diabetes. It is not well understood what causes these functional and morphologic changes that occur with age and whether in some cases these changes are beneficial or harmful. Of more immediate concern is whether substitution therapy with replacement hormones or non-hormonal agents moderate the effects of age changes in endocrine function; or increase the risk of adverse side effects in other tissues or organ systems. The previous controversy over safety of use of oral hypoglycemics drugs to treat diabetics and more recently estrogen replacement therapy in the menopause illustrate the complex and poorly understood interrelationships between functional endocrine decline and the effects of substitution or replacement therapy on target tissues or organs as well as other body systems.

The National Institute on Aging has begun to establish an integrated program of human and animal model studies on endocrine and neuroendocrine changes that occur with age. This program is aimed at characterizing and quantifying many of the changes in endocrine function that occur with age. These studies may provide the basis for understanding the causes and consequences of age related decline in endocrine function. Thus, such studies may provide the means to modify or moderate some of the pathophysiologic sequela of endocrine decline or dysfunction that occur with age.

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Extramural and Collaborative  
Research Programs

Contract Number: AG-6-2145

Contract Title: Evaluation of Comparability of the Macaca Nemestrina as a Model of Aging in Man.

Contractor: University of Washington, Seattle, Washington

Money Allocated: \$26,098.00 (Continued into FY 1977)

- Objectives:
1. To evaluate the utility of the non-human primate (M. Nemestrina) model of physical aging.
  2. To identify, collect, evaluate and provide organs and tissue for collaborative study and characterization with other investigators.
  3. To determine whether the quality and direction of physical changes are comparable to those seen in human aging.
  4. To maintain a reserve of ten aged 18-24-year old M. Nemestrina as a ready resource for later study, dependent on determination of their comparability to aging processes in the human.
  5. To provide NIA with a review of the literature on aging in non-human primates incorporating the findings from the comparative study of various physiologic systems in the 10-year old and 20-year old (M. Nemestrina) pigtail macca.

Significance for Aging Research: The study of aging requires the characterization and availability of a wide variety of strains and species of animals as well as resource materials from aged animals. To study aging changes and the differences between species of mammals as well as their relevance as models for processes of aging, the NIA must identify, characterize and develop resources that meet the needs of investigators in aging. The characterization and maintenance of an aged resource of non-human primates and the evaluation, preservation and provision of tissues and organs simultaneously retains and provides rare and unique materials that would otherwise be lost to aging research.

Proposed Course: Contract will continue for a minimum of one year and will be continued dependent on the evaluation of the model and the demand for biological materials from this resource.

Extramural and Collaborative  
Research Program

Contract Number: AG-6-2134

Contract Title: Aging, Estrogen Use, Hypertension and Myocardial Infarction

Contractor: University of California at Irvine, Irvine, California

Money Allocated: \$155,346 (FY 1977)

**Objectives:** The aim of the proposed epidemiologic study is to determine whether and to what extent estrogen usage tends to increase the risk of hypertension and myocardial infarction (MI) in a population of postmenopausal women living in a retirement community. The contract provides for the support of a retrospective study of the incidence of hypertension and MI as well as other factors which predispose to hypertension and MI in a specific and uniquely discrete population of 9,000 postmenopausal women. Cases of hypertension and MI occurring in postmenopausal women will be identified and described (morbidity and mortality). The population of identified hypertension and MI cases will be compared with an appropriate control group to determine if the case group is significantly different from that of the controls. Also, to determine the role of likely risk factors, proximity, dose and duration of drug usage in affecting hypertension and MI.

**Significance to Aging Research:** Until recently, the long-term effects of estrogens in postmenopausal women have been a matter of conjecture or largely ignored. The proposed study will provide current data on comparative risk of hypertension and MI in postmenopausal women taking estrogens and postmenopausal women who are not using them, but are otherwise at comparable risk from other causes.

The significance of this project lies in the fact that a substantial percentage of the women in the population are taking estrogen-like medications and the initial goal of the study can be completed within a two and one-half year period. At present the value of estrogen-like medication as therapy for post menopausal symptoms, for the prevention of vascular disease, and for the arrest of osteoporosis remains unproven. There is serious concern that such medication in commonly used dosage is a significant health hazard with specific reference to the three most common causes of death--heart disease, cancer, and stroke. There are not data available on the questions posed by this proposal. The population selected is uniquely constructed to permit this type of investigation. Information of this type is extremely important, since it may serve to guide the medical care of the many millions of women over the age of 50 in the United States.

**Proposed Course:** The study is planned for a minimum of two years.

Extramural and Collaborative  
Research Program

Contract Number: AG-6-2135

Contract Title: Selection and Development of an F<sub>1</sub> Hybrid Aging Rat Strain

Contractor: Charles River Breeding Laboratory, Wilmington, Massachusetts

Money Allocated: \$25,000.00

- Objectives:
1. Development of a rat model for aging research that provides a broad gene pool and maximum genetic control.
  2. Develop an F<sub>1</sub> hybrid strain that avoids pathologic lesions of body systems and endocrine tumors that are seen in most inbred strains and outbred stocks of rats currently available.
  3. Provide a strain of rat for research on aging that is less susceptible to environmental change than are in the currently available inbred strains.
  4. Provide a rat strain which has much of the generalizability to aging as an outbred stock with uniformity and predictability of pathologic and biologic characteristics of the inbred strains.
  5. Characterize the major biologic and pathologic changes over the life span of the strains selected for development.
  6. Select from among three between-strains crosses with the Fischer 344 the F<sub>1</sub> hybrid cross representative of the characteristics believed to be of greatest general applicability to the studies of aging in the rat.

Significance for Aging Research: Studies utilizing animal model systems are unique with respect to the considerations that must be exercised in the development of aging animals. Current and projected experiments in aging will require animals of defined genetic background, known biological characteristics and environmental status. Only with meticulous and exacting control of the many interacting genetic, physiologic, pathologic and environmental variables will it be possible to develop relevant animal models that may explain many of the biologic processes in aging. The F<sub>1</sub> hybrid rat model is necessary in aging research in view of the need for a rat model that is genetically defined, characterized biologically and represents a broad gene pool that permits generalizations of findings to the species as a whole.

Proposed Course: Study completed, for selection of F<sub>1</sub> Hybrid January 1979. Development of F<sub>1</sub> Hybrid strain should commence in FY 1980.

Extramural and Collaborative  
Research Program

Contract Number: AG-6-2136

Contract Title: Aging Barrier Sprague-Dawley Rat Colony

Contractor: Harlan Industries Incorporated, Indianapolis, Indiana

Money Allocated: \$83,827.00

- Objectives:
1. To meet the current demand for a commonly used stock of laboratory rat.
  2. To develop a commercial resource of aged virgin male rats on a defined diet in an essentially pathogen-free environment for investigators in aging research.
  3. To provide a ready resource of aged rats that may be used to develop techniques and procedures for studies on aging as well as pilot studies for the acquisition of preliminary data as a basis for determining feasibility for research on aging.

Significance for Aging Research: One of the primary barriers to the development of a program of studies in aging research is the lack of availability of laboratory animals in varying degrees of senescence that are representative of the aging process. To facilitate the development of aging research, standardized strains, stocks and species of animals that can be used as simulation models of aging processes must be developed in quantities that meet the needs of investigators in aging research. Since a single strain, such as the Fischer 344 cannot serve as a model for all studies, a commonly used rat of different genetic characteristics is required for a significant number of studies in aging. The Sprague-Dawley represents a distinctly different rat model for the study of aging.

Proposed Course: The colony will be maintained for a minimum of four years.

Extramural and Collaborative  
Research Programs

Contract Number: AG-3-2725

Contract Title: Contract to Breed, Rear and Maintain a Colony of Inbred Aging Laboratory Rats for Aging Research (Modified)

Contractor: Charles River Breeding Laboratory, Wilmington, Massachusetts

Money Allocated: \$150,000 FY 1977

- Objectives:
1. Meet current and projected demands for senescent laboratory rats reared on a defined diet in a specific pathogen-free environment.
  2. Establish a standing commercial resource of senescent rats on which investigators can immediately draw for aged laboratory rats.
  3. Develop baseline physiological and pathological characterization of the Fischer 344 rat over its full lifespan.
  4. Establish survival curves for laboratory rats reared specific pathogen-free behind a defined barrier system.
  5. Increase the numbers and ages of animals to be made available for studies in aging.

Significance for Aging Research: A major constraint influencing the development of aging research has been the almost total absence of an aged animal resource sufficiently characterized to meet the unique needs of aging research. The development of a colony of aging laboratory rats under this contract will significantly enhance the quality and quantity of aging research by providing aged animals that are reared in a defined environment on a standardized diet, free of pathogenic organisms, and characterized with regard to age-specific causes of death.

Basic to the development of studies in aging research in animals is a characterization of expected physiological and pathological changes that may occur over the animal's full lifespan as well as life tables that accurately reflect survival at specific ages. A primary aim of this contract is to acquire this data and make it available to investigators in aging. With this information, a reasonable comparative assessment can be made as to whether the animals, strain or stock is suitable for studies in aging. Also, within reasonable limits, numbers of animals needed for statistical significance of studies can be readily established, thus minimizing the likelihood of supporting excessive numbers of animals or too few animals for statistical significance of the study.

Currently many investigators in aging cannot acquire aged animals short of rearing the animals themselves, nor are they able to maintain aging rats under the laboratory conditions necessary to allow the animals to survive long enough to observe truly senescent change with age. Also, competent young investigators more often than not cannot support aging colonies of rats until they successfully compete for research support. Without this resource many imaginative young investigators will continue to be excluded from research in aging simply because they are unable to identify an aged animal resource which they could use in the studies they propose in aging. Thus far this colony of aged rats has served as a resource for the study of aging in more than 40 individual research projects.

~~Proposed Contract~~ Contract is to be continued for a minimum of two years with the contract becoming increasingly self-sustaining.

Extramural and Collaborative  
Research Program

Contract Number: AG-6-2811

Contract Title: Development of a Production Colony of Three Genotypes of Laboratory Mouse for Aging Research

Contractor: Charles River Breeding Laboratory, Wilmington, Massachusetts

Money Allocated: \$189,035 (FY 1977)

- Objectives:
1. Provide characterized genetically defined strains of laboratory mice reared in a defined environment for research in aging.
  2. Develop a ready commercial source of aging mice of three basic genotypes to meet the demands for aging laboratory mice.
  3. Minimize lag time for the development of studies in aging requiring aged genetically defined laboratory mice from a controlled environment.
  4. Provide the minimum number of strains of mice necessary for cross comparison and extrapolation of experimental results to a broader natural population.
  5. Develop a colony of laboratory mouse strains in which pathological processes, degenerative change, morbidity and mortality to age 24 months are largely known and predictable.

Significance for Aging Research: A lack of aged genetically and biologically defined animals reared in a controlled environment has long hampered the development of aging research, particularly in the field of immunology. With increasing frequency studies in aging research require animals of known genetic background, biological characterization and environmental status. To meet this need for strains of genetic specificity, diversity and generalizability a colony of aging mice of the inbred strains C57BL/6 BALB/c, the inbred F<sub>1</sub> hybrid of the two inbred strains was established in a barrier enclosure (SPF) at Charles River Breeding Laboratories. Profile data will be acquired on the colony and strains of animals by periodic sacrifice and necropsy.

The major significance of this contract is the development of a readily available resource of aging, genetically defined and characterized strains of laboratory mice reared in a controlled environment. The standing colony of aging mice of the three genotypes proposed under this research contract provides investigators in aging with basic genetically controlled model systems previously unavailable to most investigators in aging. This has moderated one of the primary constraining influences on the development of aging



research in animals by making available: 1) basic genetic model systems of the aging laboratory mouse for studies in aging requiring specific genetic control, 2) for study, one or several comparative animal model systems within a species, 3) an animal of known biological characterization and environmental status.

Proposed Course: Contract will continue for a minimum of three years with the contract becoming increasingly self-sustaining in subsequent years as animals are provided to investigators for research on aging.

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Contract Number: AG-5-2854

Contract Title: Aging Monkey Tissues and Organ Resource

Contractor: Washington State University, Pullman, Washington

Money Allocated: \$10,869.00 (FY 1977) continued from FY 1975

- Objectives:
1. To acquire organs and tissues from a rare resource of six (6) aged rhesus monkeys age 24 to 26 years as the animals become moribund or expire.
  2. To select and preserve organs and tissues from each of the animals that are or may be required for the study and inter-species comparison of aging and aged changes in the Rhesus monkey and other mammalian species.
  3. To bank fresh, frozen or chemically fixed and preserved tissues and organs from each of the six monkeys as they become moribund or expire.
  4. To provide selected tissues and organs on request for studies in aging.

Significance for Aging Research: The study of aging requires the availability of tissues and organs from a wide variety of strains and species of animals. To study aging changes and the comparative differences between the ordered lifespan of different species of mammals requires that the program identify and develop resources that meet the needs of the investigator in aging research. Preservation and provision of tissues and organs from aged subhuman primates, essentially expiring from natural causes, will provide a continuing resource of rare and unique materials that would otherwise be lost to aging research. The contract essentially supports the complete postmortem evaluation and preservation of tissues and organs of each of the six monkeys as they become moribund and/or expire. Postmortem protocol will require that all tissues and organs be examined, classified, and characterized. Tissues and organs from all major body systems and the integument will be selectively preserved based primarily on the requirements of the individual investigators. Other tissues and organs will be preserved by freezing or fixed in chemicals as well as preparation of slide sets of tissues from major organ systems. These materials can be provided on request for study of aging changes in the subhuman primate or comparative studies between species.

Proposed Course: Contract is to be continued for a minimum of two years.

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Contract Number: N01-AG-7-2128

Contract Title: Development of a Colony of Multigenotypic Mouse Strains

Contractor: Charles River Breeding Laboratory, Wilmington, Massachusetts

Money Allocated: \$97,000.00 (FY 1977)

Objectives: 1. To develop, define and make available the primary strains of laboratory mice that are needed to provide model systems of maximum flexibility and applicability necessary for increasingly sophisticated aging studies in biomedical research on nutrition, genetics, immunology, biochemistry, physiology and psychology.

Basic to a program of studies on comparative aging is the development and availability of a variety of species and strains of aged laboratory animals that can be used as model systems for the study of aging processes. To facilitate this program, defined and controlled animal model systems must be developed that will meet the needs of investigators in aging research. The eleven genotypes of aged mice developed under this project will enhance the ability of investigators in aging to study, trace and compare the differential aspects of aging processes by using unique attributes of mouse strains with chromosomal markers, identifying cellular characteristics, differential genetic traits and age specific morphologic and functional difference between strains and between strains  $F_1$  hybrids. Thus, this project will enhance the quality and quantity of aging research and moderate a major barrier to research on aging by making available basic mouse strains: C57BL/6, BALB/c, DBA<sub>2</sub>, B6D2F<sub>1</sub>, CBA/ca, and mouse strains with unique characteristics CBA/HT6, 129, B6C3F<sub>1</sub>, Nude, and C57BL6bg/bg, for research on aging processes.

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Research Programs

Contract Number: N01-AG-7-2118

Contract Title: Assessment of Relevant and Appropriate Models to Study  
Aging Processes

Contractor: National Academy of Sciences, National Research Council,  
Institute of Laboratory Animal Resources

Money Allocated: \$198,000.00 (through FY 1979)

Objectives: The primary objective of this project is to acquire, evaluate, develop, interpret and make available information and data on basic criteria necessary for the selection of vertebrate models to study aging processes.

The selection of relevant and appropriate animal models in the study of aging processes is complex. Selection criteria are needed to aid investigators in choosing species wisely. Unfortunately, selection and evaluation criteria for vertebrate models of aging are not currently available. Relevant information is scattered in the scientific literature and technical publications, unpublished laboratory records, and the personal commentaries of investigators experienced with particular species are additional sources of information. Therefore, the task to be accomplished by this project is to assemble and critically review relevant information on the selection of vertebrate models for research on aging, and to preface a comprehensive report containing basic criteria for their use and limitations in research on aging.

## IMMUNOLOGY AND AGING

The effects of aging on immune function have been described in experimental animals and man. Immune function over the life span of the individual increases rapidly in the early years of life, reaches a peak in young adult life, and then declines progressively. There is good evidence of a reciprocal increase in certain autoimmune phenomena and malignancies paralleling the decline in immune vigor. Since immunologically incompetent individuals are very susceptible to infections, immune dysfunction is probably one of the major sources of the health problems of the elderly. There is good evidence that immune dysfunction is to some extent involved in the pathogenesis of a variety of age-related diseases. These include cardiovascular diseases, vascular damage, kidney disease, and rheumatoid arthritis.

Much of our knowledge of the immune system in aging is limited to research on the mouse. A chief advantage of the mouse is the fact that one can work with genetically homogenous inbred strains. Secondly, the mouse is a short lived animal available in large numbers. We now have good evidence that the decline in immune capacity of the aging mouse results from deficiencies in both the immune cells and their milieu. Current studies indicate that the cellular changes responsible for senescence include: (1) a loss of certain immune cells; (2) an increase in suppressor cells; and (3) alteration in the functional efficiency of immune cells. Further studies are necessary to further elucidate the contribution of these various findings. Several studies in animals have shown that manipulation of the immune system by experimental means results in an increased life span. For example, dietary restriction profoundly affects the immune system of mice. These mice show anatomic and certain immune functional changes which suggest that the immune system may mature less rapidly under conditions of dietary restriction. The effects of dietary manipulation on immune activities, life span, and the pathogenesis of aging is under further investigation.

The research base in man is modest and primarily limited to a few studies on immunoglobulin levels and health status. An effort is underway to study the importance of genetics on immune function. Since the major histocompatibility complex represents perhaps the most important genetic system involved in antigen recognition by T-cells, it is possible that life span differences may reflect an age-related defect of the major histocompatibility complex on immune mechanisms. Preliminary results indicate that decreased immune function is associated with a decrement in a specific gene linked to immune function in women beyond the age of 70.

The National Institute on Aging is planning the expansion of its research base in immunology and aging. Questions concerning thymus function during aging, the role of suppressor cells and the substances they produce, the activity of thymic hormones and their effects upon aging, cell surface determinants and their possible relation to the appearance of age-associated diseases are all in need of further investigation. More research is required

on potential means to intervene immunologically - such as nutritional manipulation, cellular and macromolecular engineering - to delay, prevent, or even reverse the diseases of old age.

## PHARMACOLOGY AND AGING

It is quite clear that the process of aging is characterized by a deterioration of many bodily functions. Physiological impairment of the heart, blood, vessels, kidneys, digestive tract, and nervous system among other organs are prevalent in the elderly person. As the population of older citizens increases, this situation presents serious medical as well as social problems. At the present time, medical treatment of bodily dysfunction in the elderly is based primarily on experience gained in the use of drugs in people with similar diseases, but who are mainly young and mature adults. The National Institute on Aging is interested in modifying pharmacological concepts in order to illuminate the differences between the geriatric patient and the rest of the population.

Animal models have provided the cornerstone for testing and developing new drugs as well as for expanding our knowledge and understanding of drugs already in use. We have supported research directed toward characterizing in animal models changes in functions of the heart, blood vessels, and the nervous system during aging, and changes in responsiveness of these organs to drugs commonly used in the elderly. Studies have revealed that drugs used to treat disturbances in the heart beat are not as effective in old animals as they are in younger animals. Indeed such widely used drugs as lidocaine (a drug used in the intensive care unit for abnormal heart beat) and quinidine (a drug used to correct abnormal heart beat in ambulatory patients) have an entirely different spectrum of activity in older animals than in younger animals. This difference in activity may account for the numerous therapeutic failures that occur in elderly patients. These studies should provide a rational pharmacological basis for well designed clinical studies of drugs in the elderly.

The National Institute on Aging is planning the expansion of its support for research on pharmacology with emphasis on the characterization of the effectiveness of pharmacological agents by investigating the distribution, metabolism of pharmacokinetics of various drugs, the interactions of drugs, nutritional factors effecting efficacy or toxicity, and the epidemiology of drug use among the elderly. When enough information has been obtained from various models, it will be possible to develop clinical testing procedures to determine the proper use of drugs in elderly patients. The long range hope is to develop procedures in drugs that will improve the quality of life of our ever-growing aging population so that they may remain self sufficient and productive longer.

## INTERMEDIARY METABOLISM AND AGING

The effects of aging on intermediary metabolic events are varied. One feature which characterizes aging populations is a continuous decline in the capability for adaptation. This decline is evident in each individual

at many different levels of organization. It is reflected in susceptibility to disease, in the capability for avoidance of danger, in the switching on and off of vital bodily functions that typify various tissues and organs, and in the production and management of essential molecules. A general objective of this program is to understand the mechanisms which are responsible for the overall decline in the capability for adaptations and to devise means for successful intervention.

The molecular and biochemical events involved in intermediary metabolic activities occurring during maturation and aging are multifactorial and complex. For example, the adrenal cortex, the hypothalamus and the adenohypophysis are all involved in the control of glucocorticoid synthesis by the adrenal cortex. Glucocorticoid in turn is involved in the control of the metabolism of carbohydrates, lipids, and proteins. An example of impaired carbohydrate metabolism is portrayed in a defect commonly known as diabetes. Although this disease is expressed at all ages, its symptoms are particularly severe in the elderly, and by current clinical standards the disease can be anticipated to occur in virtually all the elderly. Recent experimental results indicate that a key lesson in this problem may relate those processes which determine the effectiveness of individual molecules of insulin.

Other macromolecules crucial to the integrity of function in the organism may also be affected by age. Collagen, the major structural protein in connective tissue undergoes various metabolic, chemical and physical changes with age. The National Institute on Aging has supported research on the changes with age in tendon, cartilage, bone, skin, intestine, artery and kidney. Information is currently being obtained on rates of degradation, the amount of chemical cross linking, architectural superstructure and its relationship to mechanical function. The focus is not on one type of connective tissue, but on what is common to all of them in terms of the changes which occur with age. A key question is whether the collagens and various connective tissues age simultaneously or change somewhat independently of each other.

The program also covers broadly those events dealing with energy transfer mechanisms, the metabolism and distribution of the macromolecules, such as DNA/RNA, membrane lipids, and circulating lipoproteins. The aim of the program is to quantify many of the changes occurring with age in the hope that knowledge gained may provide a basis for understanding the causes and consequences of age related decline in intermediary metabolic events. When the metabolic lesion is identified, it may be possible to investigate the reason behind its occurrence as well as likely means of intervention.

The National Institute on Aging is planning the expansion of its research base on intermediary metabolic events involved in the aging process. Program emphasis will include synthesis and metabolism of hormones, carbohydrates, lipids, proteins, lipoproteins, and other macromolecules such as DNA and RNA, collagen structure and metabolism, membrane transport and structure, and energy mechanisms which occur with aging in man and vertebrate animal models.



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Extramural Collaborative and Research Program

Contract Number: NIH-AG-76-14

Contract Title: A Study of Lymphocyte Function and Serum Immunoglobulin Concentration During the Lifespan of Individual Mice

Contractor: Cornell University, New York, New York  
(Contract Officer: Dr. Marc Weksler)

Money Allocated: \$62,883

Objectives:

To assess immune reactivity, by measuring the proliferative response of blood lymphocytes in culture and by measuring the plasma concentration of immunoglobulins. These immunological parameters will be correlated with the development of autoimmunity and longevity.

Although defects in immune function of aged mice have been repeatedly demonstrated, it has never been shown whether immune reactivity has a positive, negative or little survival value. That is, do animals with vigorous immune reactivity die early while those possessing lower reactivity survive or does immune reactivity decline with age and those with the most impaired immune function die first. Either hypothesis would result in the observed paired immune function observed in old mice.

Significance to Aging Research:

The immune system is known to undergo a marked functional decline with advancing years. Evidence exist that immune dysfunction is to some extent involved in the pathogenesis of essentially all age-related diseases. We hope to ultimately understand more about the immune processes which mediate the onset of autoimmune wasting diseases, such as cardiovascular disease, vascular damage, kidney disease rheumatoid arthritis, as well as those immunological processes which may contribute to the increased appearance of neoplasia.

Proposed Course:

The study is planned for a minimum of two additional years.

## BEHAVIORAL AND SOCIAL SCIENCES AND AGING

As indicated above, the Institute's Congressional mandate clearly includes support of behavioral and social research on aging. However, these program areas are in need of increased support in order to provide the comprehensive information necessary on the behavioral and social processes of aging. Understanding of these processes and their impact on the elderly and society should lead to advances in coping with these processes and changes. The Institute considers the improvement of the quality of life in the later years as important as increasing the number of years of life.

A major portion of the research supported in the behavioral area is concerned with the identification and description, as well as underlying mechanisms, of the intellectual and cognitive changes that occur with aging. When we have obtained accurate information about these changes and the related physiological and biological changes which may underlie them, we may be able to modify or at least develop means of coping with them. Recent research findings have cast doubt on the earlier reported findings that there is a general decline in intellectual abilities with age. At least, results based on longitudinal rather than cross-sectional studies indicate that such decline may not appear until the latter years of old age. In an NIA supported project consisting of a series of longitudinal and cross-sectional studies of intellectual development from early adulthood into old age it has been found that in abilities not involving speed of response, age changes do not occur before the late 60's. Not all subjects showed decrement even in the seventh and eighth decade. Social isolation and cardiovascular disease were found to be substantially implicated for those participants who showed decremental changes over time. Another NIA study reports that older people integrate information as well as young people and can retain as precise or accurate semantic representations of this information. However, on tests of recall, older subjects do not recall as much information as younger subjects.

Another Institute supported investigator is working towards the development of a model of declining memory in aging animals and the separation of that decline from the decline in reflexive and motor behavior that occurs with age. Such a study can provide leads for research on memory in humans.

Until recently, chronological age has been the primary criterion in assessment of work-related competency or in determining time of retirement. Attention is now turning to the possibility of functional assessment of competency. A group of NIA-supported investigators is attempting to develop a set of measures particularly suitable for older adults which will permit prediction of competent behavior in specific classes of situations. Such results will be particularly useful and relevant to non-chronological retirement criteria, or to issues such as competence to maintain independent functioning requirements for institutional support.

By the year 2020, the percentage of the population 65 years of age and older is projected to be about 16% as compared to the 10% of today. The increasing percentage and numbers of older people in the population is a phenomena that society has not had to deal with to any large degree until rather recently. What an aging of the population means and the impact of this on individuals and society are questions the answers to which should be provided by NIA supported research.

Although people in general are living longer, there are some groups in the population which apparently do not live as long as some others. This is referred to as differential life-expectancy or longevity. Those groups in the population with shorter life spans tend to be racial or ethnic minorities. The NIA is about to embark on research on the variables which have been found to have some correlation with longevity, such as socioeconomic status, racial identity and education to name a few. However, it seemed to first identify relevant data already collected in order not to duplicate efforts already underway and to determine possibilities for supplementing or "adding on" to ongoing studies. Therefore the Institute recently requested contract proposals to determine the relevant data in existence to describe and provide some evaluation of the data. A number of proposals have been received and reviewed. One will be funded before the end of Fiscal Year 1977.

Other research areas of particular interest to NIA include research on family structure and lifestyle patterns as these relate to aging, research on work and retirement, and on environmental influences on aging. Among environmental influences on aging are studies of housing and living patterns and how these affect aging, and on the impact of institutionalization upon the elderly. One presently supported project is investigating the impact of feelings of control and familiarity upon adjustment to a new environment, specifically a move into a nursing home. Through an experimental procedure the investigator is varying the level of control which individuals exert in regards to the move into the institution and measuring the impact of this in terms of adjustment to life in the nursing home correlates with the degree of involvement of the individual in arranging for admission to the home. Those with greater involvement were found to have increased activity levels, were better adjusted emotionally, and felt better physically. These individuals were also described as having greater "zest" for life.

#### MINORITY PROGRAMS

The National Institute on Aging has an interest in research that will provide greater insight into aging in various minority groups in the population and how aging in these groups may be different from aging in the white population. Our interest in this area has taken several forms - special research and training programs, and workshops.

The NIA has entered into a cooperative agreement with the National Institute of General Medical Sciences (NIGMS) to allow for NIA cooperation in the Minority Access to Research Careers (MARC) Program of NIGMS. The MARC Program provides increased opportunities for training of minority students in biomedical and behavioral sciences through three types of support, (1) a visiting scientist program, (2) faculty fellowships and (3) undergraduate training programs. The visiting scientist program provides support for visiting scientists at minority institutions, the faculty fellowships provide support for faculty at minority institutions to leave the home institution for additional training, and the undergraduate training programs provide support for students at the pre-baccalaureate level. In each aspect of the Program, basic training within a discipline is obtained. The NIA will, however, actively stimulate programs with an added emphasis in aging that will be eligible for support through the MARC Program. Increasing the pool of scientist with training in aging, especially of minority scientists, may well have to be initiated at the undergraduate level.

The NIA is also participating in the Minority Biomedical Support Program of the Division of Research Resources through which research programs at predominantly minority institutions are funded, thereby increasing the opportunities for students at these institutions to participate in biomedical research. At present, four such projects have been funded by NIA which have an emphasis on research relevant to aging.

The NIA also plans to mount a program of research which will focus on minority aging. A workshop was held recently to which NIA invited scientists from four different minority groups who are conducting research relevant to aging to obtain their advice concerning the development of a research program on minority aging. The four minority groups identified are Asian-Americans, Spanish Heritage-Americans, Native-Americans, and Black-Americans. This first workshop focussed on behavioral and social aspects of aging in minorities, but future emphasis will also be placed on biological aging in these groups. Topics for research considered thus far are reactions to stress and coping abilities, family relationships, attitudes and roles of aging, measures of quality of life, and cognitive style and aging.

NATIONAL INSTITUTE ON AGING  
ANNUAL REPORT

October 1, 1976 through September 30, 1977

Extramural and Collaborative  
Research Program

Contract Number : AG-7-2130

Contract Title : Data Sources for Studies of Differential Life Expectancy

Contractor : Enviro Control, Inc., Rockville, Maryland

Money Allocated : \$75,000

Objectives : The aim of this study is to determine the major data sources in existence that contain information relevant to studies of differential life expectancy. The contract provides for a study to describe the information in the data sources on variables, such as socioeconomic status, life style and health habits, race, and life expectancy and to evaluate the quality of this data in terms of its potential use in studies of behavioral and social correlates of life expectancy.

Significance for

Aging Research : Various groups within the population have different life expectancies, both at birth and at specific ages. Available statistical data shows the difference in life expectancy between whites and non-whites. Further examination of available statistical data indicates that life expectancy also varies with socioeconomic status as well as a number of other factors. The study to be conducted under this contract should lead to research which will provide greater understanding of the underlying causes of the difference in the life expectancy, especially in terms of information on the relationship between racial identity, socioeconomic status and longevity. Such information will ultimately provide the opportunity to work toward greater comparability of life expectancy for various groups in the population.

Proposed Course : The study is planned for a years duration.

TABLE I

DISTRIBUTION OF ECRP FUNDS  
October 1, 1976 - September 30, 1977

	NUMBER OF AWARDS	AMOUNT (\$ in thousands*)	PERCENT
RESEARCH			
Program Projects & Center Core Grants Projects (traditional)	23	6,848	44%
Scientific Evaluation Research Demonstration & Dissemination Projects	128	7,955	52%
Special Research Awards	1	63	.5%
Modified Research Career Development Award	21	323	2%
TOTAL	5	132	1%
TRAINING			
Institutional Research Training Award	178	15,401	100%
Individual Postdoctoral Research Fellowship	19	1,688	84%
TOTAL	44	312	16%
RESEARCH CONTRACTS	63	2,000	100%
INTERAGENCY AGREEMENTS	18	1,775	100%
	6	253	100%
GRAND TOTAL	270	19,409	100%

\*Funds for supplemental awards included

TABLE II  
DISTRIBUTION OF ECRP FUNDS  
Transition Quarter  
July 1, 1976 - September 30, 1976

	NUMBER OF AWARDS	AMOUNT (\$ in thousands*)	PERCENT
RESEARCH			
Program Projects	4	959	42%
Projects (traditional)	22	1,261	56%
Scientific Evaluation	1	20	1%
Modified Research Career Development Award	<u>1</u>	<u>22</u>	<u>1%</u>
TOTAL	28	2,262	100%
TRAINING			
Individual Postdoctoral Research Fellowship	11	104	100%
RESEARCH CONTRACTS	0	0	
INTERAGENCY AGREEMENTS	<u>1</u>	<u>10</u>	<u>100%</u>
GRAND TOTAL	40	2,376	100%

\*Funds for supplemental awards included

TABLE III  
DISTRIBUTION OF ECRP FUNDS BY TYPE OF AWARD  
October 1, 1976 - September 30, 1977

A

TYPE OF AWARD	NO. OF ACTIONS	AMOUNT (\$ in thousands*)	PERCENT
NEW	135	8,005	41%
COMPETING RENEWALS	22	1,546	8%
NON-COMPETING RENEWALS	96	8,951	46%
SUPPLEMENTS	14	907	5%
	<hr/>	<hr/>	
TOTAL	267	19,409	100%

B

TRANSITION QUARTER  
JUNE 1, 1976 - SEPTEMBER 30, 1976

TYPE OF AWARD	NO. OF ACTIONS	AMOUNT (\$ in thousands*)	PERCENT
NEW	14	639	27%
COMPETING RENEWALS	2	453	19%
NON-COMPETING RENEWALS	24	1,154	49%
SUPPLEMENTS	6	130	5%
	<hr/>	<hr/>	
TOTAL	46	2,376	100%

\*Includes Contracts and Interagency Agreements



TABLE IV  
SUMMARY BY MAJOR CONTENT AREA  
GRANTS/CONTRACTS

October 1, 1976 - September 30, 1977

	AMOUNT (\$ in thousands*)	PERCENT
RESEARCH		
Biology	9,105	59%
Clinical	1,070	7%
Behavioral	2,612	17%
Social	1,091	7%
Multi	1,523	10%
	<hr/>	
TOTAL	15,401	100%
TRAINING		
Biology	618	31%
Clinical	3	1%
Behavioral	403	20%
Social	108	5%
Multi	868	43%
	<hr/>	
TOTAL	2,000	100%
CONTRACTS		
Biology	1,645	93%
Clinical	55	3%
Behavioral	--	-
Social	75	4%
Multi	--	-
	<hr/>	
TOTAL	1,775	100%
INTERAGENCY AGREEMENTS	233	100%
	<hr/>	
GRAND TOTAL	19,409	100%

\*Figures include supplemental awards

TABLE V

APPLICATIONS RECEIVED

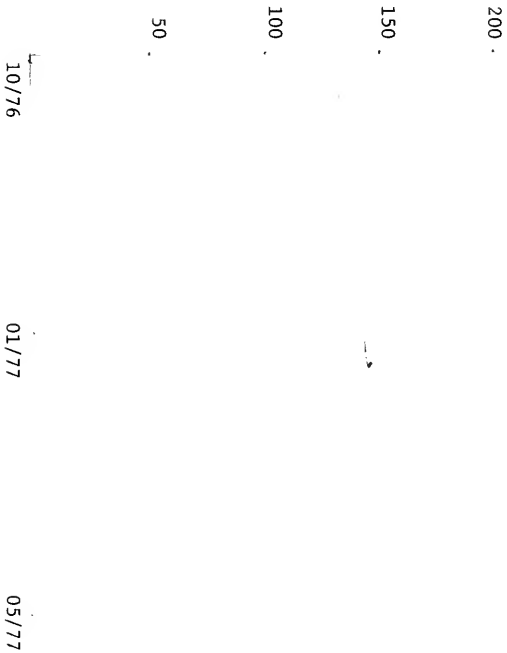
October 1976 - September 1977

		Applications Reviewed	Approval Rate/%
October 1976	Primary	95	54%
	Secondary	39	44%
January 1977	Primary	107	49%
	Secondary	39	59%
May 1977	Primary	161	58%
	Secondary	34	47%
TOTAL		195	--

APPLICATIONS REVIEWED

Figure 1

APPLICATION LEVEL, FY 1977



COUNCIL MEETINGS



Report of the Gerontology Research Center

	<u>page</u>
1. Clinical Physiology Branch	GRC/CPB-01
2. Laboratory of Molecular Aging	GRC/LMA-85
3. Laboratoty of Behavioral Sciences	GRC/LBS-133
4. Laboratory of Cellular and Comparative Physiology	GRC/LCP-185



Annual Report of the Clinical Physiology Branch, NIA  
July 1, 1976 through September 30, 1977

SUMMARY

The Clinical Physiology Branch is responsible for the maintenance of the Baltimore Longitudinal Study of Aging for the Intramural Program and conducts research on the physiological processes of aging. Where feasible, studies are conducted in man, but correlative studies are conducted in the rat and dog as well.

A summary of the current status of the Longitudinal Study shows that 1,087 participants have had a total of 6,011 visits since 1958. Of these subjects, 607 were tested 5 or more times and 169 were tested 10 or more times. There have been 168 deaths and 265 have withdrawn from the study over its 20-year history. The active sample is thus at its goal, 654 men.

The serum cholesterol levels in this upper middle-class, highly educated population has shown an interesting change in the past 6 years of the study. A drop of some 20 mg/dl has occurred in all age groups. Dietary information is available on these subjects: there has been a decided increase in the polyunsaturated/saturated fatty acid ratio. Information from the Department of Agriculture indicates that food consumption patterns in the United States also show this trend. The current study adds strength to the link between type of fatty acid ingested and serum cholesterol level by showing patterns of change in individuals who spontaneously changed their diets in response to general educational campaigns rather than as part of a rigidly controlled induced dietary change.

The kinetics of insulin distribution and destruction have been found to be different in the glucose-intolerant states of diabetes and of aging. The ratio of insulin present in tissues as compared to plasma decreased in diabetes but increased with age. Changes in obesity more closely resembled those of diabetes than of aging. These kinetic differences are evident only when groups of subjects are compared. They are unlikely to be the sole cause of the glucose intolerance of these conditions.

Further studies on the physiology of the insulinotropic hormone from the gut (gastric inhibitory polypeptide, GIP) have been completed. GIP levels in the plasma increase after both glucose and fat ingestion. Under clamped hyperglycemic conditions, fat stimulates a much larger GIP response, but the plasma insulin response to the GIP elevation is no greater after fat than after glucose. It is possible that these nutrients cause the release of two different GIP moieties.

Evidence that GIP is the major insulin secretagogue from the gut has been developed from collaborative studies in which GIP was infused in amounts which result in physiological plasma levels of the hormone. When the ambient blood glucose concentration was clamped at a mildly elevated level, infused GIP produced modest further elevation of plasma insulin. At higher glucose concentrations, GIP induced a striking insulin rise. The exogenously-administered and endogenously-released hormones thus have similar effects on the beta cell.

There is at present very little known concerning the role of human growth hormone (HGH) during adult life. Very few studies have considered the age variable and the results reported are inconsistent. In our large study on 63 healthy men aged 20 to 81 years, HGH levels were measured basally and in response to the stress of maximal physical activity on the treadmill test. Basal values (1.5 to 2.0  $\mu\text{g/ml}$ ) were unchanged by age, but post-exercise levels fell progressively from 8.5  $\mu\text{g/ml}$  in the 20's to no response at all in the 60's and 70's. These results are in contrast to those studies in which pharmacologic amounts of insulin or of arginine were given and in which age differences were not found. Thus, the total capacity of the anterior pituitary to release HGH may remain intact but the sensitivity to a physiological stimulus is apparently reduced with aging even in early adult life.

We previously showed that the progressively lower levels of total body basal oxygen consumption with increasing age was attributable to a decrease in muscle mass as measured from 24-hour creatinine excretion. The oxygen consumption of the non-muscle mass could be computed and was the same in all age groups. We have now carried out these computations on true longitudinal data on 355 men who had at least 5 sets of paired BMR and creatinine excretion data. The total body oxygen consumption data nicely confirm the cross-sectional results. However, the oxygen consumption of the non-muscle tissues increased with age longitudinally. Data were then computed separately for the 307 men who were still alive in June, 1977, and the 48 who had died. The increase in non-muscle oxygen consumption was found to be largely attributable to the group which subsequently died,  $1.9 \pm 0.5$  ml oxygen per minute per year as compared to a negligible increase of  $0.3 \pm 0.2$  in the group which remained alive. A larger number of subjects studied over a longer period of time will be required in order to investigate further the mechanism of this unexpected predictor of mortality.

Further insight has been gained into an understanding of the marked differences in sexual functioning of older men (60-79 years of age). The most active third averaged 62 sexual events per year in contrast to the lowest third which averaged less than 4 events.



Perhaps surprisingly, associated with sexual vigor were the following characteristics: strong religious commitment, farm residence prior to age 20, and a high educational and occupational level. Interestingly, the age of first coitus or of first marriage and the number of coital partners prior to age 40 were unrelated to sexual vigor in late life. Not unexpectedly, the sexually vigorous elderly were characterized by higher customary frequency of coitus in both the early and middle adult years of marriage. Of importance is the recognition that impotence in old age is by no means universally disturbing. Many men do not perceive erotic stimuli, feel no pressure to perform and are, therefore, relatively undisturbed. Most information on impotence has come from data on "patients;" that is, from that fraction of the elderly who seek help. Although the majority of our participants feel that regular sexual activity is likely to be important for health, most state that they would, nevertheless, not seek treatment for greater sexual vigor even if this were feasible.

Preliminary age differences in the blood lactic acid responses to exercise which were reported last year on 51 subjects have been strengthened in a series which now numbers 154 subjects. The time course of the blood lactic acid concentration post-exercise had previously been especially poorly-defined for older men. It is now clear that maximal blood levels are grossly underestimated for older subjects if blood samples are obtained at 30 seconds to several minutes post-exercise. Peak levels occur at 5 to 7 minutes in the old as compared to 3 minutes in young men. The levels, on the average, remain near maximal, however, in the young at 5 and 7 minutes; therefore, if a single blood sample only is to be obtained from subjects of diverse ages, it is best obtained at 6 minutes.

The first step in cellular hormone action is attachment of the hormone to a specific receptor either on the surface of or within the cell. Past work has demonstrated striking age differences in receptor number in various tissues of experimental animals. We have now also examined receptors to beta adrenergic agents (catecholamines) on the lymphocyte membranes of the participants in the Baltimore Longitudinal Study of Aging. A large reduction (50%) in the number of receptors was found, but binding affinity remained constant. These findings are consistent with the decreased concentrations with age of these receptors in other animal tissues, such as rat and dog hearts.

The decreased number of receptors in old animals could be due to decreased rate of synthesis of this specific protein or to increased rate of its destruction. The synthesis rate for the glucocorticoid intracellular receptor has now been studied in adipocytes pre-labelled with radioactive amino acids. The rate of receptor synthesis was found to be reduced by 65-70% in old cells, in agreement

with the 70% reduction in receptor concentration. This finding of a decreased synthesis rate for a specific protein may have important implications for other cellular protein synthetic processes.

Delineation of the biochemical mechanisms underlying age changes in hormone action has progressed. It was previously reported that activity of the enzyme adenylate cyclase (AC) was lost during preparation of liver cell membranes from old rats. It has now been shown that the loss of the epinephrine-sensitive AC activity is due to the removal of at least two soluble cytosol factors: GTP and a non-dialyzable, heat stable, trypsin-sensitive small protein. The loss of glucagon-sensitive AC activity is not restored by GTP but by another small protein which is heat-labile and trypsin-resistant. It is of interest that these small proteins are present in both young and old cytosol--thus, the greater loss of AC activity in the old animals during membrane preparation are not caused by differences in their cytosols.

Further advances have also been made in the studies of fat cell membrane AC. Previous work had shown that epinephrine-sensitive AC activity in human fat cell membranes required a synthetic GTP analogue. Now it has been shown that at 37° and pH 8.4 or when sufficient anion concentration is present, GTP itself is effective. Thus, the role of GTP as a mediator of fat cell AC has been shown.

The left ventricular response to afterload stress was compared in 17 normal young (mean 29 years) and 11 normal aged (mean 68 years) males. Echocardiographic measurements of left ventricular end-diastolic dimension (LVDD), left ventricular end-systolic dimension (LVSD), and velocity of circumferential fiber shortening (VCF) were made at rest and during a 30 mm Hg increase in systolic blood pressure induced by both handgrip exercise and phenylephrine infusion. At rest, there was no age difference in heart rate, LVDD, LVSD, or VCF. Both handgrip and phenylephrine induced significant changes in these parameters in both age groups but no age difference in the responses could be elicited. In order to eliminate the influence of beta-adrenergic drive, the measurements were repeated during propranolol block. While there was no age difference in ventricular response during beta-blockade at rest, phenylephrine infusion during beta-blockade induced a greater increase in LVDD in the age group ( $2.3 \pm 0.6$  mm) compared to the young group ( $0.1 \pm 0.5$  mm,  $P < 0.01$ ). This increase in LVDD in the aged group occurred despite a significantly smaller decrease in heart rate than in the young group. These data suggest that the normal aged human heart performs as well as a young heart at rest and during beta-blockade but has a greater reliance on beta-adrenergic drive during afterload stress.

In several species ranging from rat to man, the duration of mechanical activity of contraction is prolonged with age. This could be explained in part by a decrease in the ability of the relaxing

apparatus to bind calcium. To test this, microsomes were prepared from rat hearts, and calcium accumulation at all free calcium concentrations employed was found to be significantly decreased with age. In addition, contraction duration was shown to be prolonged in muscles from the same hearts. Slowing of relaxation, one of the most distinctive features of aged hearts, has thus been shown to be related to a defect in the velocity of calcium accumulation by microsomes. This is of potential clinical significance during times of stress, at high heart rates, when diminished relaxation could interfere with ventricular filling and, thus, lead to functional impairment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00001-07 CPB
--	---	---------------------------------------

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Metabolic Studies of Aging in Man.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Hershcopf	Clinical Associate	CPB NIA
	R. Andres	Chief, CPB	CPB NIA
	J. D. Tobin	Medical Officer	CPB NIA
	G. S. Raizes	Clinical Associate	CPB NIA
Other:	D. Elahi	Staff Fellow	CPB NIA
	D. D. Schocken	Clinical Associate	CPB NIA

COOPERATING UNITS (if any)

E. A. McGuire, Staff Fellow, LTB, NCI  
M. Berman, Chief, LTB, NCI

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Metabolism Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:

3.1

PROFESSIONAL:

1.9

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☐ (c) NEITHER

☐ (a1) MINORS

☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is primarily concerned with furthering our understanding of the relations between physiological aging processes and specific diseases in the elderly. The major focus is on glucose homeostasis and diabetes mellitus. Studies are directed at long-term follow-up of volunteers in the Baltimore Longitudinal Study of Aging in order to acquire actuarial data for judging the significance of varying levels of performance on the diagnostic tests for diabetes mellitus which are in clinical use. Other studies are directed at discovering the patho-physiologic mechanism underlying the age changes in performance on these tests. Serum cholesterol levels have been measured longitudinally since 1963. In addition to the effects of aging (a tendency to rise in the early adult years and to fall in old age), there have been secular trends towards a general increase in level until 1971 followed by a fall thereafter. These recent changes are coupled with nutrition changes in these subjects: lower fat intake, lower saturated fatty acid intake and higher polyunsaturated fat intake.

## Project Description:

**Objectives:** This project is primarily concerned with furthering our understanding of the relations between physiological aging processes and specific diseases in the elderly. The major focus is on glucose homeostasis and diabetes mellitus, and on aging and secular changes in serum lipids. Studies are directed at long-term follow-up of volunteers in the Baltimore Longitudinal Study of Aging in order to acquire actuarial data for judging the significance of varying levels of performance on the diagnostic tests for diabetes mellitus which are in clinical use. Other studies are directed at discovering the patho-physiologic mechanisms underlying the age changes in performance on these tests.

**Methods Employed:** Standard tests (oral and intravenous glucose tolerance, cortisone-primed oral glucose tolerance, and intravenous tolbutamide response tests) are given on a rotating basis on consecutive visits. The glucose-clamp technique has been developed for controlling the blood glucose concentration with servo-control principles. Immunoreactive assays for insulin and glucagon are used. Tracer-labeled glucose is used to measure endogenous glucose production. Kinetic modeling is done on a UNIVAC 1108 computer using the SAAM program of Berman and Weiss.

**Major Findings:** (1) Cholesterol levels in the male participants of the Baltimore Longitudinal Study of Aging have been monitored from 1963 to the present time and have been analyzed through June 30, 1977. Methodologic controls are crucial for such a study. A variety of techniques have been used to validate the methodology including the reanalysis of a sample of stored plasma (both frozen and lyophilized). Cross-sectional analysis shows the expected trends of increasing levels in the early adult years, a middle-aged plateau period, and lower levels in the aged. Analysis of longitudinal trends during the years 1963-1971 showed increases in levels which were greater than expected from the cross-sectional results. There appeared to be a secular trend toward higher levels in all age groups.

From 1971 to 1977, the trend reversed sharply. Longitudinal analysis of the data shows that cholesterol levels on the average have fallen by about 20 mg/dl. These volunteers may be characterized as an upper-middle, highly educated, socio-economic group, generally in academic, supervisory, and managerial positions. They can, thus, be expected to know of the recommendations for dietary changes in recent years that flow from the NIH and the American Heart Association.

We have obtained dietary data on our subjects by the one-week diary technique. The high educational level of the participants adds to the reliability of these data. A computer program originally developed by the Department of Agriculture and adapted at Washington University converts these food items into dietary constituents. We, thus, know that in our volunteers the percentage of calories derived from fat has dropped slightly and cholesterol intake has definitely decreased. There has also been a decided shift in the ratio of polyunsaturated to saturated fatty acid intake. This is a

reasonable explanation for at least a portion of the secular change in cholesterol levels in this population.

(2) A recent review of the literature on the effect of obesity in middle and late life on longevity has just been completed. The literature is replete on the association of obesity and major specific causes of death (atherosclerotic disease, hypertension, and diabetes mellitus being the most striking examples). Obesity is also known to be associated with gout, gall-bladder disease, osteoarthritis, and with some of the common carcinomas (breast and endometrium). Furthermore, many apparently deleterious biochemical changes accompany obesity. It was, therefore, expected that obesity would be strongly predictive of decreased longevity in longitudinal studies.

It was, therefore, surprising that in all of the studies available, with the exception of the old Society of Actuaries Study, mild and moderate obesity could not be shown to shorten life span--and this is true of both middle-aged and old age groups. Some of the studies summarized were: Framingham, Alameda County, Chicago Peoples Gas Co., Human Aging Study (NIMH), and Baltimore Longitudinal Study of Aging (NIA).

Some of the hazards of obesity have been carefully evaluated. It may be, however, that mild and moderate obesity also carries with it survival value of an unspecified nature; these beneficial effects are apparently potent enough to counteract the specific recognized hazards to longevity. The nature of this effect requires further study.

(3) The impairment of glucose tolerance by thiazide diuretics has been recognized for nearly 20 years but the mechanism of the impairment remains in dispute. A major question has been whether the effect is a direct one of the benzothiadiazines or secondary to the drug-induced potassium deficiency. We examined this in 7 normal men by studying them before and after hydrochlorothiazide administration. Potassium depletion was prevented by administration of potassium orally. The following physiological variables were assessed: glucose tolerance, beta cell sensitivity to glucose, gut cell sensitivity to glucose (GIP assay), beta cell sensitivity to GIP, and tissue sensitivity to insulin. When potassium loss was prevented, none of these variables were altered by thiazide administration. We conclude that the effects of the drug on glucose homeostasis are secondary to potassium depletion.

(4) The kinetics of unlabeled porcine insulin were studied in 69 non-diabetic male subjects age 18-83 with obesity indices of 0.93-1.51, and in 12 maturity onset diabetics age 46-78 with obesity indices of 0.95-1.56 using the glucose clamp technique. Analysis of the data using a compartmental model permitted the determination for each individual of distribution and degradation rate constants as well as a steady state tissue/plasma insulin distribution ratio ( $T/P$ ), a total plasma equivalent distribution volume ( $PEV_T$ ) and a basal systemic delivery rate (BSDR). The individuals were divided into groups allowing comparisons of the results on the basis of age, obesity index or diabetes.

The transient responses over a period of 120 minutes following a pulse of insulin show only minor differences with age, obesity or diabetes. In the steady state, however, T/P is decreased in the obese (28%) and in the diabetics (20%) but increased in the older group (16%). Since tissue insulin is both insulin bound to receptors and insulin in the interstitial fluid, changes in T/P reflect changes in *in vivo* binding to receptors, although the magnitude of the change would be modified by changes in the mass of insulin in interstitial fluid relative to plasma. BSDR increased in both the obese (18%) and the diabetics (48%), but decreased slightly with age (9%).

Four general conclusions can be drawn from the results. 1) The pattern of parameter changes seen with obesity is similar to that seen with maturity onset diabetes. 2) The decreases in T/P seen with obesity and maturity onset diabetes can not be accounted for solely by changes in fasting plasma insulin levels in these populations. 3) The pattern of changes seen in the older subjects is opposite that seen in the maturity onset diabetics suggesting that diabetes is a perturbation distinct from the normal aging process. 4) Changes in the distribution and metabolism of insulin are not large enough to be the sole cause of the alterations in glucose tolerance seen with aging, obesity or diabetes.

Significance to Biomedical Research and the Program of the Institute: The remarkable prevalence (50%) of abnormal glucose tolerance in the older population of the United States coupled with the increased morbidity and mortality of patients with true diabetes mellitus demands a delineation of the effects of aging on the patho-physiology of carbohydrate homeostasis. The tests of "abnormality" in performance levels at different ages will be certain endpoints which will develop with the passage of time: development of overt diabetes, retinopathy, coronary heart disease, and peripheral atherosclerosis; decline in creatinine clearance; mortality rate; rates of "physiological aging" in other organ systems.

Proposed Course: Longitudinal data analysis of multiple variables related to metabolic factors will continue.

#### Publications:

Davis, F.B., Estruch, M.T., Samson-Corvera, E.B., Voigt, G.C. and Tobin, J.D.: Principles of outpatient anticoagulation management. Arch. Intern. Med. 137: 197-202, 1977.

DeFronzo, R.A., Andres, R., Bledsoe, T.A., Boden, G., Faloona, G.A. and Tobin, J.D.: Test of hypothesis that rate of fall in glucose concentration triggers counter-regulatory hormonal responses in man. Diabetes 26: 445-452, 1977.

Rowe, J.W., Wands, J.R., Mezey, E., Waterbury, L.A., Wright, J.R., Tobin, J. and Andres, R.: Familial hemochromatosis: characteristics of the pre-cirrhotic stage in a large kindred. Medicine 56: 197-211, 1977.

Spector, D.A., Davis, P.J., Helderma, J.H., Bell, B. and Utiger, R.D.:  
Thyroid function and metabolic state in chronic renal failure. Ann.  
Intern. Med. 85: 724-730, 1976.



## PERIOD COVERED

July 1, 1976 to September 30, 1977

## TITLE OF PROJECT (80 characters or less)

The Gastrointestinal Mediation of Insulin Release.

## NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Elahi	Staff Fellow	CPB NIA
	G. S. Raizes	Clinical Associate	CPB NIA
	J. D. Tobin	Medical Officer	CPB NIA
	R. Andres	Chief, CPB	CPB NIA
Other:	R. J. Hershcopf	Clinical Associate	CPB NIA

## COOPERATING UNITS (if any)

D. K. Andersen, Dept. of Surgery, Duke University  
J. C. Brown, Dept. of Physiology, Univ. of British Columbia,  
Vancouver, Canada

## LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

## SECTION

Metabolism Section

## INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

## TOTAL MANYEARS:

5.7

## PROFESSIONAL:

2.6

## OTHER:

3.1

## CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS☐ (b) HUMAN TISSUES☐ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

## SUMMARY OF WORK (200 words or less - underline keywords)

This study is designed to investigate the role of the gastro-intestinal tract as an endocrine gland in the maintenance of glucose homeostasis. Among the numerous identified gut hormones, gastric inhibitory polypeptide (GIP) appears to be the candidate as the major insulin secretagogue. Three sets of studies can be added to the accumulating evidence: (1) Intravenous arginine and endogenously-released GIP are glucose-dependent insulin secretagogues when acting singly. In combination, they are additive when the concentration of arginine is low but are not when the the concentration is high. They appear to act through a common saturable mechanism. (2) Both oral glucose and oral fat induce GIP release. The time course of the GIP elevation is delayed after oral fat under steady-state hyperglycemic conditions. Plasma insulin responses are the same after oral glucose and oral fat despite the lower GIP levels after oral fat. It is possible that there are two different GIP moieties released. (3) Exogenous GIP infusion under clamped hyperglycemic conditions results in increases in plasma insulin levels and these responses are dependent upon the prevailing glucose levels. The results are comparable to those achieved in studies which cause endogenous GIP release.

## Project Description:

Objectives: This study was designed to examine generally the influence of the gastro-intestinal tract upon the insulin response to ingested glucose, and specifically the role of one or more gastro-intestinal hormones in the process of insulin secretion. The study is being applied to altered physiologic states, such as aging and obesity, as well as pathologic states of diabetes, and reactive hypoglycemia. It is well established that the response of the endocrine pancreas to ingested nutrients such as glucose is greater than its response to the same stimulus administered intravenously. The physiologic and biochemical etiology of this finding, however, has remained open to debate and investigation. Similarly, the significance of this process in pathologic states is unknown. While gastrointestinal hormones have been suggested as possible mediators of the phenomenon, until recently none has been clearly shown to play a critical role.

Methods Employed: Subjects are normal male volunteers for the new studies reported this year. The glucose clamp technique gives the investigator control of the subject's plasma glucose concentration. Insulin and GIP are measured by radio-immunoassay.

Major Findings: (1) Effects of endogenous GIP (glucose-induced) and exogenous arginine on insulin secretion. In the clamped hyperglycemic state, oral glucose causes elevation of the plasma GIP level with consequent increase in plasma insulin concentration. Also, under identical clamped hyperglycemic conditions, an intravenous arginine infusion causes an increase in plasma insulin. Since both arginine and GIP are hyperglycemia-dependent insulin secretagogues, the possibility of a common pathway of action was examined by administering them separately and together. When the arginine dose is low ( $7.5\text{g/m}^2$  surface area in one hour), the insulinotropic effect is additive to that produced by GIP released after oral glucose. When the arginine dose is doubled, then the insulinotropic effects of arginine and GIP are much less than additive. It is known that a large amount of unreleased insulin remains in the beta cell under these conditions, since other insulinotropic agents (e.g., tolbutamide and/or glucagon) will cause much higher plasma insulin levels. Thus, arginine and GIP seem to share a common saturable mechanism on insulin secretion.

(2) Effects of glucose-induced and fat-induced GIP on insulin secretion. In the clamped hyperglycemic state, oral glucose (OG) and oral fat (OF) cause a rise in plasma GIP. In the hyperglycemic studies, the initial GIP response to OF is not only delayed as compared to the GIP response to OG (25 min vs 10 min), but is only 1/3 as great at the peak (131% vs 432% over basal GIP). The plasma IRI response is similarly delayed. However, despite the lower GIP levels, the magnitude of the IRI response is nearly the same after OF and OG. These results in man are consistent with the results of other studies which show that the endogenous GIP moieties secreted after OF and OG are not identical structurally and physiologically.

The delayed time course of the GIP response to OF might be explained by the effect of fat on gastric emptying. One hypothesis to explain this delayed emptying has been that fat causes release of GIP which, through its gastric inhibitory properties, causes delay in further gastric emptying. The current studies, which show that GIP levels do not begin to increase until 25 minutes after fat ingestion, do not support this hypothesis in man and suggest that delayed gastric emptying after OF must be on another basis.

(3) Effects of GIP infusions on insulin secretion. The finding that after oral glucose plasma levels of GIP rise and that the plasma insulin concentration rises nearly identically is evidence that GIP is the important insulin secretagogue ("incretin") from the gut. But, other gut hormones, even unidentified ones, could also be playing a role as incretins. To test further the role of GIP, studies were conducted in collaboration with Dr. John Brown at Vancouver. Exogenous GIP infusions were administered under hyperglycemic clamp conditions. The infusion rate was 6.7  $\mu\text{g/kg}$  body wt per minute, carried out over a 90 minute period. Steady-state hyperglycemia was maintained in one set of studies at 54 mg/dl above basal and in another set at 143 mg/dl above basal. The GIP infusion resulted in plasma levels of GIP within the physiologic range. In the mildly hyperglycemic state, GIP infusion induced a definite but relatively small increase in plasma insulin (<50% increase). In the more severe hyperglycemic state, GIP infusion induced a striking (5 fold) increase in plasma insulin. It is, therefore, unnecessary to postulate another gut hormone to explain the incretin effects of food ingestion--GIP satisfies this requirement. Exogenously administered GIP and endogenously released GIP are insulin secretagogues and both show dependence upon the prevailing plasma glucose concentration.

#### Significance to Biomedical Research and the Program of the Institute.

The high prevalence of altered glucose tolerance in aging and obesity, as well as the high incidence of adult-onset diabetes mellitus, require further understanding of factors which contribute to this process. In addition, an understanding of similarities and differences in the pathophysiology of the various categories of glucose intolerance provides the hope for more and improved methods of treatment. Pathological states associated with alterations in gastro-intestinal hormones are only superficially understood currently, and further investigation of these hormone systems adds greatly to a new area of medical knowledge.

Proposed Course: Further studies of the interactions of insulin secretagogues on the beta cell will be conducted. The effect of age on these mechanisms remains a primary focus. The kinetics of GIP distribution and metabolism will be investigated.

#### Publications:

Andres, R. and Tobin, J.D.: Endocrine Systems. In Finch, C.E. and Hayflick, L. (Eds.): Handbook of the Biology of Aging. New York, Van Nostrand Reinhold Co., 1977, pp. 357-378.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00004-04 CPB
PERIOD COVERED July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (80 characters or less) Ethanol Metabolism: The Effect of Age on the Pharmacokinetics of Ethanol in Man.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:  Other:	J. D. Tobin R. Andres E. A. McGuire A. H. Norris	Medical Officer Chief, CPB Staff Fellow Chief, HPS  CPB NIA CPB NIA LTB NCI CPB NIA
COOPERATING UNITS (if any) R. E. Vestal, VA Hospital, Boise, Idaho E. Mezey, Dept. of Medicine, Baltimore City Hospitals, Baltimore, MD		
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch		
SECTION Metabolism Section		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this study was to examine under conditions of acute ethanol administration the effect of age on: (1) the kinetics of distribution and elimination of ethanol, (2) posterior pituitary function and free water clearance, (3) psychomotor and cognitive performance. Ethanol concentrations in the blood reach slightly higher levels in older subjects even though standardized amounts are administered to all subjects. This is attributable to the change in <u>body composition</u> (loss of cellular mass) with <u>aging</u> . The rate of <u>ethanol metabolism</u> does not change with age.		

## Project Description:

Objectives: The purpose of this study was to examine under conditions of acute ethanol administration the effect of age on: (1) the kinetics of distribution and elimination of ethanol, (2) posterior pituitary function and free water clearance, (3) psychomotor and cognitive performance.

Methods Employed: Ethanol was administered intravenously as a 15% solution in a dose of 400 mg/m<sup>2</sup> surface area/min over 60 minutes following an overnight fast. Blood ethanol concentrations were measured by gas liquid chromatography at fixed intervals during and for 4 hours after the infusion. The SAAM-26 computer program is being used to develop a compartmental model to describe the kinetics of distribution and elimination.

Major Findings: This project is reported only to record the publication of the results.

Significance to Biomedical Research and the Program of the Institute: The changes in body composition with aging can influence pharmacokinetic estimates of drug metabolism. These studies point to the fact that clinical pharmacologic studies in aging need to be interpreted with full knowledge of the physiological alterations that accompany aging.

Proposed Course: This project is completed and is hereby terminated.

## Publications:

Vestal, R.E., McGuire, E.A., Tobin, J.D., Andres, R., Norris, A.H. and Mezey, E.: Aging and ethanol metabolism. Clin. Pharmacol. Ther. 21: 343-354, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00005-04 CPB
PERIOD COVERED July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (80 characters or less) Hypothalamic-hypophyseal Responsiveness and Aging.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	R. Andres	Chief, CPB
	J. D. Tobin	Medical Officer
Other:	None	CPB NIA CPB NIA
COOPERATING UNITS (if any) J. H. Helderman, Dept of Nephrology, Southwestern Medical School, Dallas, Texas G. L. Robertson, Chief, Endocrine Sec., VA Hospital, Indianapolis, Indiana		
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch		
SECTION Metabolism Section		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MICROS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) These studies are designed to ascertain the effect of aging hypothalamic-hypophyseal secretion of the <u>antidiuretic hormone</u> , <u>arginine vasopressin</u> (AVP), in response to: (1) overnight dehydration, (2) hypertonic saline infusion and (3) intravenous ethanol infusion. These standardized tests of both negative ( <u>ethanol</u> ) and positive ( <u>hypertonic saline</u> ) stimuli to the <u>hypothalamic-hypophyseal</u> axis showed: (1) with <u>aging</u> there is an enhanced sensitivity of AVP secretion to hyperosmolality; (2) the inhibitory effects of ethanol on AVP do not change with age during the first 30 minutes of the infusion, then there is a paradoxical loss of the inhibition in older subjects; (3) the transient effect of ethanol in the aged is probably due to the increase in serum osmolality induced by the ethanol, thus secondarily causing a counter-effect of AVP secretion. The increased sensitivity of this endocrine mechanism with age is probably an important factor in the known susceptibility of older patients to the development of serious <u>hyperosmolality syndromes</u> .		

## Project Description:

**Objectives:** These studies are designed to ascertain the effect of aging on hypothalamic-hypophyseal secretion of the antidiuretic hormone, arginine vasopressin (AVP), in response to: (1) overnight dehydration, (2) hypertonic saline infusion and (3) intravenous ethanol infusion. From such studies, mechanisms of aging changes will be described and some of the clinical problems in salt and water conservation in aging individuals may be explained.

**Methods Employed:** The hormone arginine vasopressin was measured by radioimmunoassay of acetone-extracted plasma utilizing the Glick-1 (G1-1) anti-serum. The technique is highly specific as it can distinguish AVP from all other naturally synthesized and secreted posterior pituitary peptides. Samples of plasma obtained during the various perturbations were rapidly frozen and assayed at a later date.

In all studies, subjects across the age spectrum were carefully selected to eliminate those who had cardiac or renal diseases and those taking alcohol or drugs. They refrained from fluids overnight. Plasma samples obtained just prior to a perturbation thus represented the AVP response to mild overnight dehydration. In one group of studies, 3% NaCl was infused by vein over 2 hours following a 20 minute basal period in the recumbent position. Plasma samples were obtained during the infusion for plasma AVP, sodium, and osmolality. Urines were collected before and after the infusion for the measurement of sodium and osmolality. The goal of these studies is to provide a hyperosmolar stimulus which is recognized by the hypothalamus and leads to posterior pituitary release of antidiuretic hormone.

In another group of studies, 15% (v/v) ethyl alcohol was infused intravenously at the rate of  $400 \text{ mg/m}^2/\text{min}$  over one hour. Blood samples for AVP, ethanol, sodium, and osmolality were obtained at intervals over 5 hours. As ethanol has been implicated in diminishing or abolishing secretion for AVP, it provided a negative stimulus to test the hormonal axis.

Urine and plasma measurements of osmolality and volume allowed computation of osmolar clearance and of free water clearance which quantify the physiologic response to the AVP present in the blood.

**Major Findings:** The results of the infusions of hypertonic saline and of ethanol on plasma AVP levels and on renal responses to AVP are in press. The only additional finding to be reported is that of paradoxical hypersecretion of AVP after ethanol infusion (normally an inhibitor to AVP release) when the infusion induces nausea. Two young and two old men in the group became severely nauseated during the infusion. Each had acute elevations of AVP at the time of onset of nausea. Two of the subjects had distinct waves of nausea, each accompanied by spikes of AVP elevation. The important caveat is that in the assessment of AVP secretion, nausea must be avoided. Furthermore, the data suggest possible connections between the neural emetic center and the hypothalamus.

Significance to Biomedical Research and the Program of the Institute: There are clinical indications that the aged individual has a diminished ability to maintain salt and water homeostasis. Studies performed here in the past have examined the end organ phenomena that pertain to salt and water balance. Analysis of the central mechanisms involved is required to understand fully the impact of aging on the ability to maintain the internal milieu. Such understanding may alter clinical decisions about fluids and medications prescribed for the elderly patient.

Proposed Course: The project is completed except for the publication of the findings above.

Publications:

Helderman, J.H., Vestal, R.E., Rowe, J.W., Tobin, J.D., Andres, R. and Robertson, G.L.: The response of arginine vasopressin to intravenous ethanol and hypertonic saline in man: the impact of aging. J. Geron., in press.



## PERIOD COVERED

July 1, 1976 to September 30, 1977

## TITLE OF PROJECT (80 characters or less)

Age Effect on Intrinsic Cardiac Muscle Regulation and Neural  
Control of Heart and CirculationNAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER  
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Spurgeon	Staff Fellow	CPB, NIA
	F. C. P. Yin	Clinical Associate	CPB, NIA
Others:	J. P. Froehlich	Medical Officer	LMA, NIA
	N. W. Shock	Scientist Emeritus	NIH

## COOPERATING UNITS (if any)

M. L. Weisfeldt, Dir., Div. Cardiology, Johns Hopkins Univ.  
J. L. Greene, Asst. Prof. of Medicine, JHU

## LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

## SE

Cardiovascular Section

## INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

## TOTAL MANYEARS:

0

## PROFESSIONAL:

0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS☐ (b) HUMAN TISSUES☒ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

## SUMMARY OF WORK (200 words or less - underline keywords)

This project is a broad multifaceted program which attempts to characterize and quantify the changes in cellular and organ physiology and pharmacology which occur with advancing age. A major goal of the program is to project the findings of one discipline in terms of the meaning to the whole system by measuring a spectrum of indices of cardiac performance at several levels of integration on the same tissue samples. Thus one is better able to assign some relative value to each measured reaction or cellular event in terms of the overall control of cardiac function. Neural, pharmacological, and physiological techniques are employed extensively from a functional level, while biochemical assay techniques are used to identify cellular change.

Combined into Projects Z01 AG 00025-01 and Z01 AG 00026-01 CPB.

Project Description:

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Combined into Projects Z01 AG 00025-01 CPB and Z01 AG 00026-01 CPB.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00025-01 CPB												
PERIOD COVERED July 1, 1976 to September 30, 1977														
TITLE OF PROJECT (80 characters or less) Calcium Accumulation in Cardiac Microsomes from Young Adult and Senescent Rats														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 35%;">E. G. Lakatta</td> <td style="width: 40%;">Chief, Cardiovascular Section</td> <td style="width: 15%;">CPB, NIA</td> </tr> <tr> <td></td> <td>J. P. Froehlich</td> <td>Medical Officer</td> <td>LMA, NIA</td> </tr> <tr> <td>Others:</td> <td>H. A. Spurgeon</td> <td>Staff Fellow</td> <td>CPB, NIA</td> </tr> </table>			PI:	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA		J. P. Froehlich	Medical Officer	LMA, NIA	Others:	H. A. Spurgeon	Staff Fellow	CPB, NIA
PI:	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA											
	J. P. Froehlich	Medical Officer	LMA, NIA											
Others:	H. A. Spurgeon	Staff Fellow	CPB, NIA											
COOPERATING UNITS (if any)														
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch														
SECTION Cardiovascular Section														
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224														
TOTAL MANYEARS: .8	PROFESSIONAL: .4	OTHER: .4												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords)  The project is designed to measure the ability of the <u>cardiac relaxing system (sarcoplasmic reticulum)</u> from <u>young adult and aged hearts to accumulate calcium</u> . This data will further <u>elucidate the mechanism of prolongation of contraction</u> in the aged heart.														

## Project Description:

Objectives: In several species ranging from the rat to man, the duration of mechanical activity of contraction has been demonstrated to be prolonged. This could be explained in part by a decrement in the ability of the relaxing apparatus to bind calcium in the aged heart. The relaxing apparatus can be isolated and in vitro calcium accumulation measured. Mechanical parameters (duration of contraction) can also be measured in trabeculae isolated from the same heart.

Methods: (1) Microsomes are prepared by the standard method of Hiragawa and Schwartz. Steady state velocity of calcium binding over a range of free calcium concentrations is measured in the presence of oxalate.

(2) Mechanical measurements are made in a standard myograph apparatus.

(3) These measurements are also made in young adult and aged canine hearts.

Major Findings: (1) Velocity of calcium accumulation at all free calcium concentrations employed is significantly diminished in microsomes from aged rat hearts.

(2) Contraction duration is prolonged in muscles from the same heart used to prepare microsomes studied in (1).

(3) The studies in the dog demonstrate the same aging trend, but an additional number must be performed to reach a firm conclusion.

Significance to Biomedical Research and to the Program of the Institute: The results are significant to biomedical research because they encourage further studies to elucidate the mechanism for the difference. If this can be determined another "block" can be added to our currently incomplete "wall" of knowledge regarding the relaxation process in heart per se. The results are significant to the program of the institute in that one of the most distinctive features of aged hearts of many species, including man, is a slowing of relaxation. This is of potential clinical significance during times of stress, at high heart rates, when diminished relaxation could interfere with ventricular filling and lead to enhanced dyspnea and functional impairment.

Proposed Course: (1) The present study measures only steady state rates of calcium accumulation. Our next goal is to acquire equipment necessary to measure the rapid burst of activity that precedes the steady state rate, as this may be more tightly

coupled to relaxation of the tissue. Additional studies will examine the relationship between these rapid rate kinetics and relaxation in the tissue following manipulations that are known to alter the relaxation process in both systems. Such studies will enable us to become "front-line soldiers" in the "war" to understand the nature of the relaxation process in heart muscle. These studies will be performed in close collaboration with Dr. Froehlich of the Molecular Biology Section, LMA.

(2) To measure the effect of catecholamines (protein-kinase) in phosphorylating these microsomes. These experiments are very pertinent to aging research since an age difference in response to catecholamines in mechanical studies of cardiac muscle has been demonstrated.

#### Publications:

Froehlich, J. P., Lakatta, E. G., Beard, E., Spurgeon, H. A., Weisfeldt, M. L., and Gerstenblith, G.: Studies of sarcoplasmic reticulum function and contraction duration in young adult and aged rat myocardium. J. Mol. Cell. Cardiol. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 AG 00026-01 CPB</div>
PERIOD COVERED <div style="text-align: center;">July 1, 1976 to September 30, 1977</div>		
TITLE OF PROJECT (80 characters or less) <div style="text-align: center;">Age Associated Alterations in Response to Catecholamines</div>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:   Others:	F. C. P. Yin H. A. Spurgeon E. G. Lakatta G. S. Roth	Clinical Associate Staff Fellow Chief, Cardiovascular Section Staff Associate
		CPB, NIA CPB, NIA CPB, NIA CPB, NIA
COOPERATING UNITS (if any) <div style="text-align: center;">M. L. Weisfeldt, Dir., Div. Cardiology, Johns Hopkins Univ.</div>		
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch		
Cardiovascular Section		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS: <div style="text-align: center;">.8</div>	PROFESSIONAL: <div style="text-align: center;">.75</div>	OTHER: <div style="text-align: center;">.05</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <span><input checked="" type="checkbox"/> (a) HUMAN SUBJECTS</span> <span><input type="checkbox"/> (b) HUMAN TISSUES</span> <span><input type="checkbox"/> (c) NEITHER</span> </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <span><input type="checkbox"/> (a1) MINORS</span> <span><input type="checkbox"/> (a2) INTERVIEWS</span> </div>		
SUMMARY OF WORK (200 words or less - underline keywords) <p>Previous work from this laboratory has demonstrated that when compared to the <u>young adult rat myocardium</u>, <u>myocardium from aged rats</u> exhibits a <u>diminished inotropic response to catecholamines</u>. The (chronotropic) response to these agents has now been expanded to both <u>dog</u> and <u>man</u>.</p>		

## Project Description:

Objectives: (1) To measure the inotropic effect of catecholamines in isolated rat myocardium with measurements of  $\beta$ -receptor numbers in the same heart. (2) To expand (1) into the canine model. (3) To measure the chronotropic response to catechols in intact dogs. (4) To measure the chronotropic response to catechols in man.

Methods: (1) and (2) Trabeculae carnae are equilibrated in standard myograph and the enhancement of force production in response to infused catecholamines is measured. The remainder of the heart is used to prepare a crude microsomal preparation, in which the number of  $\beta$ -receptors is measured utilizing the Al-prenolol method.

(3) The increment in heart rate in response to a range of concentrations of Isoproterenol is measured in unanesthetized dogs before and after vagal blockade. The heart rate response to atrial pacing is measured. On a separate occasion, with the dog in the awake state, the response to a maximal dose of Isoproterenol is repeated.

(4) The response to infusion of Isoproterenol in increasing doses is measured in awake resting man. The limit increment in a given subject is 25 bpm.

Major Findings: (1) Though only a small number of studies have been completed, the diminished mechanical response in muscles from hearts of aged rats is reproducible, and simultaneous measurements of  $\beta$ -receptors indicate that their maximal number is reduced in aged hearts.

(2) The number of dogs sacrificed to date is too limited to formulate tentative conclusions.

(3) The chronotropic response to Isoproterenol is significantly diminished in the aged beagle vs. the young adult beagle. This age difference was noted at maximal and all submaximal doses, and was present in both the awake and anesthetized dogs. The age difference persisted after vagal blockade. No age difference resulted from direct atrial overdrive pacing. This indicates that no age difference is present at the effector level.

(4) At all doses of Isoproterenol studied, the men in the aged group (65-80 yrs.) demonstrated a significant diminished increment in heart rate. No difference was present in resting heart rate. The results of (3) and (4) are in manuscript form and ready to submit for publication.

Significance to Biomedical Research and the Program of the Institute: The decreased response to all forms of stress - exercise, hypoxia, etc. may be explained at least in part by the diminished response to catecholamines, the physiological mediators of the stress response. Since the diminished stress response is one of the most notable changes that occurs in an aged individual, studies to elucidate the mechanism of the altered response are of major import. These studies require collaboration between molecular biologists and physiologists.

Proposed Course: The response to catecholamines is mediated via a cascade of events, each of which can be isolated and studied. These steps are as follows: (a)  $\beta$ -receptor, (b) cyclic AMP - GMP - phosphodiesterase, (c) protein kinase - phosphorylation, and (d) enhancement of calcium flux into the cell. The feasibility of measuring  $\beta$ -receptors, cyclic nucleotides and protein kinase, and the mechanical response in single heart is currently being considered.

Publications:

Gerstenblith, G., Lakatta, E. G., and Weisfeldt, M. L.: Age changes in myocardial function and exercise response. Prog. Cardiovasc. Dis. 19: 1-21, 1976.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01* AG 00009-03 CPB																
PERIOD COVERED July 1, 1976 to September 30, 1977																		
TITLE OF PROJECT (80 characters or less) Age Changes in the Mechanical Properties of the Cardiovascular System																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PERSONNEL ENGAGED ON THE PROJECT																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 60%;">H. A. Spurgeon</td> <td style="width: 30%;">Staff Fellow</td> <td style="width: 10%;">CPB, NIA</td> </tr> <tr> <td></td> <td>F. C. P. Yin</td> <td>Clinical Associate</td> <td>CPB, NIA</td> </tr> <tr> <td>Others:</td> <td>P. T. Thorne</td> <td>Head, Technical Development</td> <td>OSD, NIA</td> </tr> <tr> <td></td> <td>E. G. Lakatta</td> <td>Chief, Cardiovascular Section</td> <td>CPB, NIA</td> </tr> </table>			PI:	H. A. Spurgeon	Staff Fellow	CPB, NIA		F. C. P. Yin	Clinical Associate	CPB, NIA	Others:	P. T. Thorne	Head, Technical Development	OSD, NIA		E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
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Others:	P. T. Thorne	Head, Technical Development	OSD, NIA															
	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA															
COOPERATING UNITS (if any) W. Milnor, Prof. of Physiology, Johns Hopkins University M. L. Weisfeldt, Dir., Div. Cardiology, JHU																		
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch																		
S. Cardiovascular Section																		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 33%;">OTHER:</td> </tr> <tr> <td style="text-align: center;">1.2</td> <td style="text-align: center;">1.2</td> <td></td> </tr> </table>			TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	1.2	1.2											
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																
1.2	1.2																	
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords) <p>             Mechanical properties of heart muscle determine to a great extent the way in which biochemical events in the cell are transduced into muscle tension and ultimately into development of pressure in the heart. Changes in the <u>mechanical properties</u> of the <u>aged heart</u> could account for the observed changes in cardiac performance as determined by a variety of indices. This program uses <u>vibration techniques</u> to measure the components of a <u>cardiac</u> and <u>vascular muscle</u> which determine the overall "<u>stiffness</u>," and measures the <u>age</u> induced effect of stiffness produced by regulatory events of both <u>neurohumoral</u> and <u>mechanical</u> origins. As the major elements of cardiac control, these events, and their interaction with the mechanical properties of the heart form the basis for <u>cardiac control</u>.           </p>																		

## Project Description:

Objectives: The objectives of this study are (1) to quantitate the influence of age on the dynamic stiffness in isolated muscle from both rat and dog hearts; (2) to induce a high impedance state with aortic banding and to measure resulting secondary changes of dynamic stiffness in cardiac muscle; (3) to measure the effect of the level of activation of a muscle on dynamic stiffness; and (4) to measure the aortic input impedance in intact rats.

Methods: (1) Isolated superfused trabeculae carneae from adult and senescent rats are clamped in an apparatus which constrains the muscles to an isometric length. Using techniques developed for vibration analysis, the stiffness of the sample of cardiac muscle is determined as a stress/strain ratio by subjecting the isometric muscle to sinusoidal length perturbations, typically of 0.022 mm, at frequencies from .001 to 100 Hz. The muscle is caused to contract isometrically during the study, enabling computation of the changes in dynamic stiffness associated with the contractile process. The resulting tension, which varies in step with the sinusoidal length changes imposed, is the strain produced stress, and by definition represents the total stiffness of the muscle. Stiffness is also measured at different levels of activation induced by altering the bath perfusate and by adding pharmacologic agents. This study is also performed on canine hearts.

(2) In a large number of rats a band is placed on the aorta and the study in (1) is repeated in hearts from these animals in which the duration of banding ranges from 1 to 8 months. The execution of tedious preliminary experiments enables band placement such that approximately 15% hypertrophy will occur, thus simulating the extent of hypertrophy in the aged heart.

(3) The measurement of impedance is accomplished in much the same way, except the perturbation used is the contraction produced pressure of the rat heart. The pressure in the ascending aorta is measured by a catheter introduced via the carotid artery, while blood flow is measured by electromagnetic flowmeter. Using Fourier analysis to break the two signals into harmonic components and comparing the ratio of pressure to flow of each harmonic yields impedance information. Both this and the stiffness technique use perturbations and measure the result of these perturbations.

Major Findings: (1) The initial study showed that resting stiffness increased by about 25% with a similar increase in active contractile stiffness in senile rat myocardium as compared to young adult animals. It was further shown that the aged hearts

move up a stiffer function curve which appears to be valid for both passive and active stiffness. Thus, the increased resting tension noted in past studies from this laboratory appears to be the result of an increase in the intrinsic stiffness of the resting muscle, while the development of active contractile tension, which appears undiminished in the old muscles, is actually accomplished with a stiffer muscle. This suggests that muscle from aged hearts develops the requisite amount of tension with a reduced amount of muscle fiber shortening as compared to young rat myocardium. The number of canine muscles studied is still too few to arrive at a tentative conclusion regarding age alterations in dynamic stiffness in this model.

(2) An initial group of experiments on muscles from banded animals has been concluded and the results are currently being analyzed.

(3) An initial group of experiments on the influence of the level of activation on dynamic stiffness has been completed and the results are currently being analyzed.

(4) Measurement of aortic impedance in unanesthetized rats has been accomplished and no age difference was demonstrated. However, the effect of anesthesia and the reliability of the resulting measurements made in the rather small rat aorta remain in doubt. Feasibility studies to measure aortic input impedance in awake trained dogs are currently being performed.

Significance to Biomedical Research and the Program of the Institute: The implications of the completed study have rather wide impact. First, the indication of increased stiffness in both passive and active states of the aged heart muscle suggests the age related change of this important measure occurs not only in the passive supportive structure of the muscle, but implies changes in the active contractile elements as well. Second, the suggestion that the old heart stiffness vs. tension curve exhibits a steeper slope, and that this slope increases with age in both the passive and active state indicates a fundamental change in the muscle with age. Third, although it is widely held that the old heart is incapable of extended performance under stress, the efficiency of the aged heart may actually be increased. Assuming a series model, tension developed is the product of distance the contractile element shortens and the stiffness of the element. For tension to remain relatively unchanged across age, the amount of shortening required may decrease by as much as 25%. A similar shift in the aortic impedance would further modify the efficiency of cardiac performance. Although tension development fails to decrease markedly with age, the tension developing mechanism might well decrease, and the magnitude of that decrease may prove greater than

suspected because of the "masking" effect of increased stiffness.

Proposed Course: (1) To continue studies of dynamic stiffness in muscles from hypertrophied animals. (2) To continue studies investigating the influence of the level of activation on dynamic stiffness. (3) To measure dynamic stiffness and the influence of vasoactive substances in aortic muscle from young adult and aged rats. (4) To attempt to measure aortic input impedance in awake unanesthetized dogs.

Publications:

Spurgeon, H. A., Thorne, P. R., Yin, F. C. P., Shock, N. W., and Weisfeldt, M. L.: Increased dynamic stiffness of trabeculae carnea from senescent rats. Am. J. Physiol. 232: H373-H380, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00010-04 CPB
PERIOD COVERED July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (80 characters or less) Echocardiographic Assessment of the Left Ventricle and Mitral Valve in Aging Man		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:  Others:	F. C. P. Yin T. Guarnieri H. A. Spurgeon E. G. Lakatta	Clinical Associate Clinical Associate Staff Fellow Chief, Cardiovascular Section CPB, NIA CPB, NIA CPB, NIA CPB, NIA
COOPERATING UNITS (if any) N. J. Fortuin, Asst. Prof. of Medicine, Johns Hopkins Univ. M. L. Weisfeldt, Dir., Div. Cardiology, JHU G. Gerstenblith, Cardiology Fellow, University of Miami		
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch		
SECTION Cardiovascular Section		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS:  .5	PROFESSIONAL:  .4	OTHER:  .1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Management, evaluation, and control of the <u>aged heart</u> require knowledge of the changes in regulation and control of the normal old heart. This study investigates, <u>in man</u> , the effect of age on the primary mechanism of cardiac control exerted by the <u>sympathetic nervous system</u> , and measures the response of hearts studied across a <u>spectrum of ages</u> (18-88) both at rest and after <u>induced work loads</u> using <u>noninvasive measures</u> of <u>heart size</u> and <u>performance</u> provided by <u>echocardiography</u> .		

## Project Description:

Objectives: Noninvasive techniques are employed in man to measure the function of the left ventricle while it is subjected to a known amount of stress. The rationale is that diseased or failing hearts may appear to function normally when not subjected to undue stress, but reveal abnormalities when subjected to even a moderate degree of stress. Previous animal experiments from this laboratory have demonstrated that the aged but otherwise healthy stressed heart has certain functional impairments. Comparable information in man is lacking. Utilizing modern techniques, it is possible to examine this question in man. By comparing the performance of normal (by standard criteria) young and aged hearts both unstressed and during the imposition of a controlled degree of stress, one is able to assess the degree of impairment in heart function due to the aging process.

Methods: Echocardiograph assessment of the aging heart includes two major projects. The first study characterized the left ventricle, aorta and mitral valve motion at rest in a normal adult population. The second study utilizes the echocardiogram to measure left ventricular function during imposition of a stress. A predetermined increase in peripheral blood pressure is induced sequentially by isometric handgrip exercise or by infusion of an epinephrine-like drug (phenylephrine). Measurements are made before and after temporary blockade of the sympathetic nervous system achieved by infusing the beta-adrenergic blocking drug propranolol. Electrocardiograms and blood pressure are recorded simultaneously with the echocardiogram. Left ventricular function is assessed by measuring changes in the diastolic and systolic dimensions and velocity of shortening of the endocardium during the various interventions.

Major Findings: The results of Project I demonstrate that the left ventricular thickness increases as a function of age while cavity size and calculated velocity of shortening and ejection fraction were unchanged. The mitral valve E-F slope (closure) was also significantly diminished with age. The results of Project II demonstrate that when beta-adrenergic tone is eliminated, the left ventricular cavity dilates in response to phenylephrine more in the aged than in the adult population. These results are now in manuscript form and ready to be submitted for publication.

Significance to Biomedical Research and the Program of the Institute: Information concerning the functional impairment, if any, of the aged but otherwise normal, healthy human heart as well as the sensitivity of the regulatory mechanisms is critical to understanding one aspect of the aging process. Results of this study may enable one to (1) evaluate and make better recom-

mendations to elderly persons regarding the advisability of specific exercise activities, (2) help predict those people who are likely to develop significant heart disease as they age, and (3) better understand the overall changes in cardiac regulation as an individual ages.

Proposed Course: The echocardiogram will continue to be a useful tool in measuring responses to stress and pharmacological agents. Proposed studies include investigation of the feasibility of utilizing the echocardiogram during semi-supine bicycle exercise. Assessment of cardiac performance in women will also be considered for comparison with the data gathered on the aged male.

Publications:

Gerstenblith, G., Frederiksen, J., Yin, F. C. P., Fortuin, N. J., Lakatta, E. G. and Weisfeldt, M. L.: Echocardiographic assessment of a normal aging population. Circulation 56: 273-278, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00024-01 CPB																
PERIOD COVERED July 1, 1976 to September 30, 1977																		
TITLE OF PROJECT (80 characters or less) Response to Cardiac Glycosides in Senescent Dogs and Isolated Canine and Rodent Cardiac Muscle																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">T. Guarnieri</td> <td style="width: 40%;">Clinical Associate</td> <td style="width: 10%;">CPB, NIA</td> </tr> <tr> <td></td> <td>E. G. Lakatta</td> <td>Chief, Cardiovascular Section</td> <td>CPB, NIA</td> </tr> <tr> <td></td> <td>H. A. Spurgeon</td> <td>Staff Fellow</td> <td>CPB, NIA</td> </tr> <tr> <td>Others:</td> <td>J. P. Froehlich</td> <td>Medical Officer</td> <td>LMA, NIA</td> </tr> </table>			PI:	T. Guarnieri	Clinical Associate	CPB, NIA		E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA		H. A. Spurgeon	Staff Fellow	CPB, NIA	Others:	J. P. Froehlich	Medical Officer	LMA, NIA
PI:	T. Guarnieri	Clinical Associate	CPB, NIA															
	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA															
	H. A. Spurgeon	Staff Fellow	CPB, NIA															
Others:	J. P. Froehlich	Medical Officer	LMA, NIA															
COOPERATING UNITS (if any)  M. L. Weisfeldt, Dir., Div. Cardiology, Johns Hopkins Univ																		
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch																		
SE Cardiovascular Section																		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224																		
TOTAL MANYEARS: <div style="text-align: center;">.5</div>	PROFESSIONAL: <div style="text-align: center;">.5</div>	OTHER:																
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords) Investigation of: (1) Direct effect of <u>cardiac glycosides</u> in isolated cardiac muscle from rats and dogs. (2) Effect of <u>cardiac</u> <u>glycosides</u> on <u>electrophysiology</u> and <u>mechanical performance</u> in <u>intact dogs</u> . (3) Effect of cardiac glycosides and <u>Na<sup>+</sup>K<sup>+</sup> ATPase</u> in cardiac muscle from canine species.																		



## Project Description:

Objectives: The objectives of this study are to characterize the effect of age response to cardiac glycosides both in the intact organism and in isolated heart muscle.

Methods: (1) Intact dogs.

(a) Unanesthetized awake dogs are given rapid bolus injection of acetyl-strophanthidin. This is a rapid acting glycoside. Doses are repeated in 5  $\mu\text{g/kg}$  increments each 30 minutes until the end point of the study - ventricular tachycardia ensues. The dose required to produce this toxic effect is compared in both young adult and senescent beagles. Serum levels of the drug are monitored prior to each dose.

(b) Anesthetized dogs are instrumented with a catheter tipped micro-monometer placed in the left ventricle and a pacing catheter inserted into the right ventricle. The dose response curve obtained in a given dog in the awake state is repeated in the anesthetized state. The inotropic response of pressure and rate of pressure development are measured at a given paced rate; in addition toxic effects on the ECG are simultaneously measured.

(c) Beta blockade. On a third day (nonsuccessive), part (b) is repeated in the presence of propranolol, a beta-adrenergic blocking drug. Thus in a given dog, electrical responses are measured in the awake state, and both electrical and mechanical responses are measured in the anesthetized state. When the dog is subsequently sacrificed at a later date, muscle is removed from the heart and the direct effect of the glycoside on the muscle is measured (see below).

(2) Isolated muscle. Trabecular muscles are isolated from hearts of the rat and dog and the responses to ouabain (rat) and acetyl-strophanthidin (dog) are measured and compared to the response to other inotropic interventions (increased both calcium and paired stimulation).

Major Findings: (1) Preliminary results indicate that there is no age difference in the dose of A.C.S. necessary to induce ventric tachycardia in the awake dog. An additional number of studies in anesthetized dogs is needed to complete this project and this data is currently being generated.

(2) In isolated rat cardiac muscle, there is a marked significant decrement in the inotropic response to ouabain, while no age difference exists in response to calcium or paired pacing. These results suggest that the receptor for ouabain (Na-K ATPase)

and/or steps intermediate between the receptor and effector are altered with age. The number of studies on isolated dog muscle of both mechanical and Na-K ATPase response is too few for analysis at the present time.

Significance to Biomedical Research and the Program of the Institute: The age difference demonstrated in the direct effect of ouabain on isolated cardiac muscle indicate that the sarcolemmal receptor sites or ion exchange pumps are altered with age. Since cardiac glycosides are widely used in treatment of heart failure, the demonstration of an age difference in response to these agents in several species would be of obvious clinical import.

Proposed Course: (1) To complete the studies in the canine model.

(2) To further study the mechanism of the age differences in response, both in rat and dog cardiac muscle, should age differences in the dog be demonstrated.

(3) To consider the feasibility of measuring the effect of glycosides in aged man utilizing the echocardiogram and systolic time interval to monitor the response.

Publications:

Lakatta, E. G.: Perspectives on the Aged Myocardium. In Cristofalo, V., Roberts, J., and Adelman, R. (Eds.): Advances in Experimental Medicine and Biology. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF</b> INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00011-05 CPB
PERIOD COVERED July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (80 characters or less)  Hormones, hormone receptors, and aging I. Aging and hormone-sensitive adenylate cyclase.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  PI:        M. S. Katz                      Clinical Associate (resigned June 30, 1977)    CPB    NIA R. I. Gregerman              Chief, Endocrinology Section  OTHER:    None		
COOPERATING UNITS (if any)  Department of Surgery Baltimore City Hospitals		
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch		
SECTION Endocrinology Section		
INSTITUTE AND LOCATION NIA, GRC, Baltimore, Maryland 21224		
TOTAL MANYEARS: 4.0	PROFESSIONAL: 1.5	OTHER: 2.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  These studies deal with influences of <u>age</u> on the biochemistry of <u>hormone-sensitive adenylate cyclases</u> in a variety of tissues. The purpose of these studies is to explore the mechanisms of age-related alterations of <u>hormone</u> responsiveness and biological membranes. The work utilizes preparations of materials from rats and human (liver, fat).		

## Project Description:

Objectives: The first step in the action of a hormone is its reversible combination with a specific cell component, the receptor, followed by initiation of some other biochemical event which eventually leads to expression of a biological effects. Recently it has become possible to quantitate the receptors for hormones, while the next biochemical step has for several years been under study in a number of laboratories. For many hormones this second step involves the formation of the compound cyclic adenosine-3'-5' monophosphate (cAMP) from ATP by the action of the enzyme, adenylate cyclase (AC). Many cell functions are under the control of this key enzyme and its messenger, cAMP. Aging is known from many studies to be associated with altered hormonal responsiveness, but the precise mechanisms have only recently started to receive attention. Our investigations are designed to provide new information in this area. Both the hormone receptors related to adenylate cyclase and the enzyme itself are integral components of the cell's outer membrane. Our work, therefore, involves certain problems related to the effects of aging on the membranes, per se.

Methods Employed: Tissues used include human and rat fat and liver from rat. Isolated fat cells, tissue homogenates, the preparation of cell membranes involve standard methods of collagenase digestion, density gradient centrifugation, etc. Adenylate cyclase is measured by conversion of  $^{32}\text{P}$ -ATP to  $^{32}\text{P}$ -cAMP followed by quantitation of the product by isolation on columns. Use of  $^3\text{H}$ -cAMP and  $^{14}\text{C}$ -cAMP allows precise quantitation of recoveries during incubations and isolation. Receptors for hormones are quantitated with labeled ligands, usually antagonists.

Major Findings: 1) Altered AC Activity During Preparation. Our previous work showing that in rat liver homogenates epinephrine responsive AC increases 100% (2-fold) in old animals while their membranes become unstable has now been published. During the course of that work, loss of AC was noted during preparation of crude membranes. Quantitation of this phenomenon has now been studied in detail and has been submitted for publication. The mechanism of the loss of enzyme activity has also been studied in detail and is also about to be submitted. In brief, preparation of membranes results in loss of 20-75 percent of activity for both epinephrine and glucagon responsive adenylate cyclases. These losses are not due to mechanical losses during isolation, but to altered enzyme responsiveness in the remaining membranes. Loss is to some extent sex dependent, but is most marked for epinephrine sensitive enzyme in 24 month old animals.

2) Cytosol Factors in the Loss of Adenylate Cyclase During Preparation of Membranes from Homogenates. Loss of epinephrine-sensitive adenylate cyclase activity of liver is due to removal during membrane preparation of at least two soluble cytosol factors: one is almost certainly the guanine nucleotide, GTP. However, GTP accounts for only part of the loss. Another factor is non-dialyzable, heat stable, and destroyed by trypsin. This suggests that the substance is a small protein. Concentrated cytosol restores nearly all of the lost epinephrine-sensitive activity. The loss of glucagon-sensitive enzyme is

less marked than that of epinephrine but is still considerable ( $\pm 50$  percent). This lost activity can also be restored by cytosol. GTP does not restore activity. The glucagon effective material is non-dialyzable, heat-labile and trypsin resistant, suggesting that it is a protein but one which is different from that which affects epinephrine sensitive enzyme. Both old and young cytosol appear to be equally effective for both enzymes, suggesting that the greater loss of enzyme in the old animals is not related to changes of the cytosol factors and their concentration. These observations are being prepared for publication.

3) Characterization of Human Fat Cell Adenylate Cyclase. Previous work from our laboratory suggested a guanine nucleotide requirement for human fat cell epinephrine-responsive adenylate cyclase. The synthetic GTP analogue, GMP-P(NH)P was shown by us to allow expression of epinephrine sensitive cyclase activity. However, GTP itself was inhibitory. Subsequent work now shows that when enzyme assays are performed at  $37^\circ$  instead of  $30^\circ$  and at slightly higher pH than before (8.4 vs. 7.6), GTP has the capacity to enhance epinephrine sensitive human fat cell adenylate cyclase. The temperature effect is probably through phase transitions in the lipid membranes. In comparative studies no such effect is seen with rat fat cell enzyme, although the inhibitory effect normally expected by GTP disappears. The results with human enzyme show for the first time that GTP is involved as a mediator of fat cell adenylate cyclase. This material was presented at the 3rd International Conference on Cyclic Nucleotides.

4) Role of Anions in Adenylate Cyclase Activity. Further studies of this area reveal that GTP acts like GMP-P(NH)P, i.e., becomes a direct activator of fat cell cyclase when sufficient concentrations of anions are present. These observations further involve GTP in fat cell cyclase regulation.

5) Quantitation of  $\beta$ -adrenergic Receptors in Liver and Their Relationship to Age-Related Changes of Adenylate Cyclase. We have explored two techniques for quantitating  $\beta$  receptors. Both use labelled antagonists,  $^3$ H-dihydroalprenolol and  $^{125}$ I-hydroxybenzylpindolol. Quantitation in liver is needed in order to help explain mechanisms of the observed age change of  $\beta$  receptor, i.e., epinephrine sensitive-adenylate cyclase. To date neither method has provided adequate estimates for either homogenates or membranes, but our efforts are continuing.

Significance to Biological Research and the Program of the Institute. These studies are illuminating the basic mechanisms of aging and altered hormone responsiveness.

Proposed Course of the Project: Efforts will be made to study further the age-related membrane changes associated with alterations of epinephrine-sensitive adenylate cyclase in liver. The nature of the cytosol factors will be examined and their isolation attempted. Efforts to quantitate  $\beta$  receptors will continue.

Publications:

Cooper, B., Partilla, J. S., and Gregerman, R. I.: Enhanced Activity of Hormone-Sensitive Adenylate Cyclase during Dietary Restriction in the Rat. Dependence on Age and Relation to Cell Size. Journal of Clinical Investigation 59: 467-474, 1977.

Kalish, M. I., Katz, M. S., Pineyro, M. A., and Gregerman, R. I.: Epinephrine and glucagon-sensitive adenylate cyclases of rat liver during aging. Evidence for membrane instability associated with increased enzymatic activity. Biochimica et Biophysica Acta (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00012-05 CPB																									
PERIOD COVERED July 1, 1976 to September 1977																											
TITLE OF PROJECT (80 characters or less)  Hormones, hormone receptors and aging. II. Aging and hormone responsiveness.																											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																											
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">G. S. Roth</td> <td style="width: 40%;">Research Chemist</td> <td style="width: 10%;">CPB</td> <td style="width: 10%;">NIA</td> </tr> <tr> <td colspan="5"> </td> </tr> <tr> <td>OTHER:</td> <td>W. C. Chang</td> <td>Visiting Fellow</td> <td>CPB</td> <td>NIA</td> </tr> <tr> <td></td> <td>D. D. Schocken</td> <td>Clinical Associate</td> <td>CPB</td> <td>NIA</td> </tr> <tr> <td></td> <td>R. I. Gregerman</td> <td>Chief, Endocrinology Section</td> <td>CPB</td> <td>NIA</td> </tr> </table>			PI:	G. S. Roth	Research Chemist	CPB	NIA						OTHER:	W. C. Chang	Visiting Fellow	CPB	NIA		D. D. Schocken	Clinical Associate	CPB	NIA		R. I. Gregerman	Chief, Endocrinology Section	CPB	NIA
PI:	G. S. Roth	Research Chemist	CPB	NIA																							
OTHER:	W. C. Chang	Visiting Fellow	CPB	NIA																							
	D. D. Schocken	Clinical Associate	CPB	NIA																							
	R. I. Gregerman	Chief, Endocrinology Section	CPB	NIA																							
COOPERATING UNITS (if any)  None																											
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch																											
SECTION Endocrinology Section																											
INSTITUTE AND LOCATION NIA, GRC, Baltimore, Maryland 21224																											
TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.0	OTHER: 0.2																									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																											
SUMMARY OF WORK (200 words or less - underline keywords)  This project is mainly involved in relating <u>age</u> , the <u>intracellular</u> and <u>cell surface receptors</u> for <u>hormones</u> and the biological responsiveness of hormone-sensitive tissues.																											

## Project Description:

Objectives: Altered responsiveness to hormones is an important manifestation of the aging process. The present study is attempting to determine the various mechanisms by which such changes occur. One key step in these investigations is an understanding of hormone receptors and the mechanisms of their control during senescence.

Methods Employed: Whole animals, isolated tissues, and defined cell populations in short term culture are used. In addition, white blood cells are obtained from Longitudinal Study participants in accordance with Human Research Committee guidelines. Hormone receptors, located either on the cell surface or intracellularly, are studied qualitatively and quantitatively by measuring binding of labeled steroids, catecholamines and other hormones to tissues, cells and subcellular fractions. Affinity chromatography is used to isolate hormone receptors for measurement of synthetic and degradative rates as well as purification for preparation of antisera and immunochemical titration. Hormonal control of various cellular metabolic processes such as nutrient transport and utilization are measured by standardized techniques. Macromolecular biosynthetic processes are also assessed.

Major Findings: 1) One class of cell membrane hormone receptors, those for  $\beta$ -adrenergic agents (catecholamines), have been examined for the first time in cells taken directly from aging man. Although binding affinity remains constant, concentrations of these receptors in lymphocyte membranes progressively decrease with increasing age. The magnitude of this reduction is about 50% between 20 and 80 years of age. Thus aging changes in hormone receptor levels, similar to those which result in altered responsiveness in animals, appear to occur in humans. These results are consistent with decreased concentrations of  $\beta$ -adrenergic receptors in various aged animal tissues, including those of rat and dog hearts as previously investigated in our laboratory.

Studies of rat heart  $\beta$ -adrenergic receptor and aging have been continued in collaboration with the Cardiovascular Section. Correlations between loss of receptors and loss of mechanical responsiveness during aging have now been made in individual groups of heart preparations. In addition, the loss of receptors during aging has been found not to be due to age-related differential recovery of sarcolemma, since recovery of  $^3\text{H}$ -wheat germ agglutinin labeled material is comparable in both young and old membrane preparations.

Further insight into the mechanisms responsible for loss of  $\beta$ -adrenergic receptors during aging has been obtained through use of certain guanine nucleotides. These compounds are capable of re-activating receptors which have been temporarily rendered non-functional due to previous association with hormone. When aged rat heart  $\beta$ -adrenergic receptors are assessed in the presence of such nucleotides, many receptors which are not detected in untreated preparations can be measured. Such *in vitro* nucleotide treatment of young and old heart membranes in preliminary experiments seems to reduce the apparent age-related loss of functional receptors. Since several groups have now reported increased levels of circulating catecholamines during aging in



man, one possible mechanism for apparent age losses of  $\beta$ -adrenergic receptors may be through "desensitization" due to increased hormonal exposure.

2) An isolated adipocyte system from Wistar rats of various ages is being used to answer several questions regarding post-mitotic cellular aging and hormonal responsiveness. Preliminary results suggest that the ability of  $\alpha$ -adrenergic agents to stimulate glucose utilization is decreased in aged cells. Thus,  $\alpha$ -adrenergic receptors may also change during senescence.

The rates of glucocorticoid receptor synthesis and turn-over are also being measured in these cells. We previously showed that the apparent concentration of these intracellular receptors is progressively reduced during aging. Glucocorticoid receptors are labeled by exposing adipocytes to radioactive amino acids for short periods in vitro. These receptors can be selectively removed from other labeled proteins by affinity chromatography using glucocorticoid hormones coupled to insoluble Sepharose beads. Preliminary results suggest that although protein content and overall rates of protein synthesis are equal in mature and old adipocytes, the rate of glucocorticoid receptor synthesis is reduced 65-70% in aged cells. This reduction is consistent with our previously reported 70% reduction in receptor concentration. Thus, one possible mechanism for the apparent age loss of steroid receptors may be a decreased rate of receptor synthesis in senescent cells. If confirmed, these findings also suggest that the synthesis of selective groups of proteins may be altered during aging.

Significance to Biomedical Research and the Program of the Institute: A prime manifestation of the aging process is a reduced capacity for adaptation. This decline is intimately linked with alterations in the ability to respond to hormones. We thus seek an understanding of the mechanisms by which hormone action changes during aging. One phenomenon involved in these changes appears to be a loss of certain intracellular and membrane hormone receptors. Such loss impairs cellular recognition of hormonal signals and dampens response to these stimuli. The present study is attempting to define those processes which control hormone receptor levels, and seeks ways to control receptors and physiologic responsiveness during senescence.

Proposed Course of the Project: 1)  $\beta$ -adrenergic receptors will continue to be examined in lymphocytes from Longitudinal Study participants. Correlations between age changes in receptor levels and biochemical responses such as control of glucose utilization and activation of adenylate cyclase will be attempted. In addition, longitudinal subjects already studied will be examined again on future visits to determine the extent of individual age changes in  $\beta$ -adrenergic receptors and response.

Studies of these receptors will also be continued in rat heart in collaboration with the Cardiovascular Section. Particular emphasis will be placed on mechanisms of receptor control such as by nucleotides, thyroxine and other hormone treatments, and by exercise. Other cell surface receptors including those for  $\alpha$ -adrenergic agents and dopamine will be examined during aging, since decreased responsiveness to these hormones occurs with increased

age. The latter will be performed in collaboration with the Laboratory of Behavioral Sciences.

2) Studies of glucocorticoid receptor synthesis and degradation will be continued. Additional controls to be performed include elution of receptors from affinity absorbants and subsequent physicochemical characterization, purification and preparation of antisera to receptors for eventual immunochemical titration, and cell free receptor synthesis and degradation experiments. Emphasis will also be placed on elucidating various possible steroid receptor control mechanisms. Transplantation and parabiosis experiments will be performed to distinguish between aging processes intrinsic to cells and those intrinsic to the cellular environment. Our ultimate goal will be control of various hormone receptor and responsiveness levels during aging.

#### Publications:

Roth, G. S.: Reduced glucocorticoid binding site concentration in cortical neuronal perikarya from senescent rats. Brain Research 107: 345-354, 1976.

Roth, G. S. and Livingston, J. N.: Reductions in glucocorticoid inhibition of glucose oxidation and presumptive glucocorticoid receptor content in rat adipocytes during aging. Endocrinology 99: 831-839, 1976.

Roth, G. S.: Altered Biochemical Responsiveness and Hormone Receptor Changes During Aging. In Behnke, J. and Finch, C. (Eds.): A New Look at Biological Aging. Am. Institute of Biological Sciences (in press).

Roth, G. S.: Hormonal Receptor and Responsiveness Changes During Aging: Genetic Modulation. In Harrison, D. H. (Ed.): The Genetics of Aging. Original Article Series of the National Foundation (in press).

Schocken, D. D. and Roth, G. S.: Reduced  $\beta$ -adrenergic receptor concentrations in aging man. Nature 267: 856-858, 1977.

Schocken, D. D. and Roth, G. S.: Age-Associated Loss of Beta Adrenergic Receptors from Human Lymphocytes. In Roberts, J., Cristofalo, V. J. and Adelman, R. C. (Eds.): Explorations in Aging II. New York, Plenum Press (in press).

Roth, G. S., Schocken, D. D. and Chang, W. C.: Changes in Hormone Action During Aging: The Role of Target Tissue Hormone Receptors and Neuroendocrine Regulatory Mechanisms. In Mariois, M (Ed.): Institute de la Vie World Congress on Aging. North Holland, Amsterdam (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF          INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER  Z01 AG 00013-03 CPB																				
PERIOD COVERED <b>July 1, 1976 to September 30, 1977</b>																						
TITLE OF PROJECT (80 characters or less)  <b>Hormones, hormone receptors, and aging. III. Aging and the human male reproductive system.</b>																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																						
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">S. M. Harman</td> <td style="width: 40%;">Senior Investigator</td> <td style="width: 10%;">CPB NIA</td> </tr> <tr> <td colspan="4" style="height: 10px;"></td> </tr> <tr> <td>OTHER:</td> <td>P. D. Tsitouras</td> <td>Visiting Associate</td> <td>CPB NIA</td> </tr> <tr> <td></td> <td>C. E. Martin</td> <td>Senior Investigator</td> <td>CPB NIA</td> </tr> <tr> <td></td> <td>R. I. Gregerman</td> <td>Chief, Endocrinology Section</td> <td>CPB NIA</td> </tr> </table>			PI:	S. M. Harman	Senior Investigator	CPB NIA					OTHER:	P. D. Tsitouras	Visiting Associate	CPB NIA		C. E. Martin	Senior Investigator	CPB NIA		R. I. Gregerman	Chief, Endocrinology Section	CPB NIA
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COOPERATING UNITS (if any)  None																						
LAB/BRANCH <b>Gerontology Research Center, Clinical Physiology Branch</b>																						
SECTION <b>Endocrinology and Human Performance Sections</b>																						
INSTITUTE AND LOCATION <b>NIA, GRC, Baltimore, Maryland 21224</b>																						
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SUMMARY OF WORK (200 words or less - underline keywords)  <p>           This project is an attempt to define influences of <u>age</u> on the male reproductive system, especially the production of <u>male hormones</u> (<u>testosterone</u>, <u>dihydro-testosterone</u>), the trophic hormones, <u>LH</u>, and <u>FSH</u>, and the latters' responses to <u>LH-RH</u>. Correlations between hormone levels, <u>hormone binding</u> to plasma proteins and the interrelations of these factors with <u>aging</u> are being made in the <u>longitudinal study subjects</u>.         </p>																						

Objectives:

A. Background- The sex hormones are important biological mechanisms for control of development and function throughout the life span. The hypothalamus, regulates sexual function by secreting the hormone LHRH into the blood supply of the pituitary gland. LHRH stimulates the pituitary to release the gonadotrophins FSH and LH, which control gonadal function. In the male LH acts to promote production of the male sex hormone, testosterone, by secretory (Leydig) cells, while FSH acts together with testosterone to cause sperm production in the testis tubules. Testosterone, in addition, is responsible for developing and maintaining male secondary sex characteristics (body and facial hair, muscle mass) and plays an important role in sex drive. Testosterone also "feeds back" on the hypothalamus and pituitary to regulate secretion of the gonadotrophins.

B. Current knowledge- Recent work by a number of investigators has considerably clarified the nature of the physiologic events surrounding senescence of the male reproductive system. Several studies have shown that there is considerable loss of Leydig cell function beginning in men in their 50's. Circulating blood levels of total and especially "free" (non-protein bound) testosterone decreases along with an increase in circulating LH and FSH. These findings suggest decreased gonadal function as the primary event in producing decreased testosterone secretion with advancing age. Pituitary function appears to be better preserved, but the response of elderly men to LHRH injection may be slightly subnormal. An additional finding is that the levels of estrogens (female hormones) appear to increase in aging men which in turn increases the amount of testosterone binding protein activity of the plasma and so further diminishes the amount of "free" (i.e. available) testosterone. One group reported decreased testicular volume and decreased facial, pubic, and axillary hair in their older subjects. To date, however, none have attempted to correlate individual testosterone levels with changes in secondary sex characteristics, libido, onset of prostatism, or development of cardiovascular disease (long known to be more frequent in males). Studies of seminiferous tubular function in terms of quantity and quality of sperm production are sketchy. All available studies suffer from the limitation of being cross-sectional rather than longitudinal. If reproductive system functions (such as plasma testosterone) influence longevity, the population studied might become progressively skewed with advancing age.

C. Present study- Objectives are (1) to provide information on gonadal function in the aging human male with regard to gonadotrophin secretion and pituitary gonadotrophin reserve, testosterone production and testicular responsiveness to gonadotrophins, and semen production (2) to elucidate the relationships between pituitary, Leydig cell, and seminiferous tubular function in the aging male, with the object of determining the site (or sites) of failure of the reproductive system and (3) to correlate behavioral and health variables such as libido and prostate disease with levels of endocrine function in the aging human male.

Methods employed: (1) Plasma gonadotrophins are being assayed using a double antibody radioimmunoassay (2) Plasma testosterone, dihydrotestosterone, and estradiol are measured using a charcoal type radioimmunoassay (3) Assay results are analyzed by a computerized method. (4) Semen analysis is performed using standard techniques for determining number and quality of sperm. (5) The free fraction of testosterone in plasma samples is estimated using DEAE cellulose "mini-columns". (6) Clinical samples are obtained in two ways: (a) Longitudinal Study subjects are given endocrine stimulation tests with multiple serum samples taken at intervals, providing both baseline measurements and data regarding ability of the system to respond when stimulated. These tests are made with LHRH for pituitary gonadotrophin reserve and hCG for Leydig cell secretory capacity. In addition, a series of semen samples are being collected from those subjects able to cooperate. Libido is estimated from interview. (b) Freeze dried plasma samples, taken from the same longitudinal subjects in past years, will be analyzed in order to compare gonadotrophin and androgen levels with libido scores previously obtained and with other health parameters.

Major Findings: To date 70 Longitudinal Study subjects over the age range of 20 to 89 have participated in the study. There are at least 10 men in each decade except for the age range 20-29 where a shortage of subjects exists.

Measurements of FSH and LH both basal and after LHRH stimulation have been completed for all but a few of the subjects and preliminary analysis shows an upward trend in basal levels of both gonadotrophins with age.

The majority of subjects have also had their samples assayed for testosterone, but a problem with non-linearity of response and interassay variance may require that these samples be reassayed. Pilot assays for dihydrotestosterone, estrone, and estradiol have now been conducted successfully, but subject samples have not yet been tested for these steroids.

A simple reliable method for determination of free vs protein bound testosterone has been developed in our laboratory using small DEAE cellulose columns. A manuscript describing this method has been accepted for publication.

Fewer than 1/3 of the subjects participating have complied with the request to donate sperm samples for study. For this reason data on semen quality is insufficient to draw firm conclusions; however, it would appear that there is a tendency in the oldest (over 75) subjects toward the appearance of azoospermia (absence of sperm) in the ejaculates. A trend toward diminution of testicular size with age is also apparent.

No attempt has yet been made to correlate hormone measurements with such other variables as sexual activity, coronary or prostate disease, or psychological test scores, but Dr. Martin has updated the sexual history on nearly all participants.

#### Significance to Biomedical Research and the Program of the Institute:

Quantitative information on the decrease in male reproductive function with age which correlates hormonal, psychological, and other variables is sorely lacking. What proportion, if any, of the increased incidence of impotence and decreased sex drive in aging men is endocrinologic, and therefore

potentially reversible, is not known. Data regarding the interactions of age, reproductive physiology and sexual behavior will add a new and useful parameter to the GRC longitudinal study, and may suggest both specific therapy for sexual dysfunction and new avenues of investigation into the nature of the aging process.

Proposed Course: Recruitment of a number of new young volunteers age 20-29 has been proposed in order to complete the first cross-sectional cycle of this longitudinal study. The second cycle (2 year intervals) of the study will begin in November 1977. Efforts to streamline and improve methodology will continue. Should a significant correlation between age, sex hormones and other variables (such as sex drive) appear, a short term (6 mo.) therapeutic trial of testosterone replacement in a limited number of subjects may be proposed.

Publications:

Harman, S. M. and Danner, R. L. Rapid measurement of protein bound testosterone using batch elution from ion exchange columns. J. Clin. Endocrinol. Metab. (in press).

Harman, S. M. Clinical Aspects of Aging of the Male Reproductive System. In Schneider, E. (Ed.): The Aging Reproductive System. New York, Raven Press, 1977, pp. 29-58.

Amatruda, J. M., Harman, S. M., Pourmotabbed, G., and Lockwood, D. H. Depressed plasma testosterone and fractional binding of testosterone in obese males. J. Clin. Endocrinol. Metab. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF</b> INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00023-02 CPB																				
PERIOD COVERED July 1, 1976 to September 30, 1977																						
TITLE OF PROJECT (80 characters or less)  Hormones, hormone receptors and Aging, IV. Aging and Leydig cell function.																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																						
<table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 30%;">S. M. Harman</td> <td style="width: 20%;">Sr. Invest.</td> <td style="width: 10%;">CPB</td> <td style="width: 10%;">NIA</td> </tr> <tr> <td colspan="5" style="height: 20px;"></td> </tr> <tr> <td>OTHER:</td> <td>P. D. Tsitouras</td> <td>Visiting Associate</td> <td>CPB</td> <td>NIA</td> </tr> <tr> <td></td> <td>G. S. Roth</td> <td>Research Chemist</td> <td>CPB</td> <td>NIA</td> </tr> </table>			PI:	S. M. Harman	Sr. Invest.	CPB	NIA						OTHER:	P. D. Tsitouras	Visiting Associate	CPB	NIA		G. S. Roth	Research Chemist	CPB	NIA
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COOPERATING UNITS (if any)  None																						
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SECTION Endocrinology Section																						
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SUMMARY OF WORK (200 words or less - underline keywords)																						
<p>This project is an attempt to develop an animal model system for studying the effects of aging on <u>Leydig Cell</u> function (testosterone production) in the male. In vitro preparations of partially purified <u>Leydig Cells</u> are being studied for <u>alterations with age in hormone metabolic function</u>.</p>																						

## Project Description:

Objectives: The testicular Leydig cell is the source of the male hormones, principally testosterone, which produce masculine secondary sex characteristics and maintain male sex drive both in man and animals. It is apparent that in aging humans these cells produce less male hormone in response to gonadotrophin stimulation. A similar situation has been described for the rat by several investigators.

The testicular secretion of testosterone is under the control of the pituitary gonadotrophin, LH. In addition to stimulating increased secretion of testosterone by pre-existing Leydig cells, LH recruits undifferentiated interstitial cells located in the tissues of the testis and causes them to become steroid secreting cells. The mechanism of LH stimulation involves its binding to specific receptors on the outer membrane of the cell followed by activation of the membrane-bound enzyme, adenylate cyclase, which in turn catalyzes the conversion of ATP to cyclic AMP (cAMP). cAMP accumulation is responsible for multiple effects within the cell, including increased synthesis and/or activation of enzymes which convert cholesterol to sex steroid hormones. Our present goal is to define an animal model for the aging Leydig cell and to investigate the biochemical and cytological basis of age-related defects of this cell population.

Methods employed: Rats of various ages were subjected to Leydig cell stimulation with injections of hCG (an available gonadotrophin similar in action to LH) using 2 different protocols: a long term (3 day) series of injections to measure the ability of the animal to differentiate Leydig cells from the inactive interstitial cell population, and a short period of stimulation (3 hrs) designed to measure responsiveness of Leydig cells already present. Blood samples taken at various intervals were analyzed for testosterone by radioimmunoassay.

Major Findings: The rat appears to differ from the human in that aging rats develop a full testosterone response to hCG after an initial lag period of approximately an hour. This in turn suggests a deficiency of intrinsic stimulatory activity of rat gonadotrophins with age.

Significance to Biomedical Research and the Program of the Institute: This investigation is an effort to define the cytological and biochemical defects in a population of hormone responsive cells showing decreased function with age. Such a study should help to elucidate the nature of tissue and cellular aging.

Proposed Course: Investigations are continuing using *in vitro* preparations of rat Leydig cells to detect changes with age in receptor binding, cyclase activation or, testosterone synthetic activity which might not have been revealed by the less sensitive *in vivo* methods. Since the intact rat does not show a decreased testosterone response to exogenous gonadotrophin as does the human, it may be necessary that species other than the rat (possibly a non-human primate) be similarly investigated if a system analagous to aging of the human reproductive mechanism is to be found.



Result to date indicate a delayed response to hCG by Leydig cells of aging rats. Currently rat Leydig cells are being isolated in vitro and examined, for synthesis of testosterone, the number and character of hCG receptors, adenylate cyclase activation, and the activity of enzymes required for sex steroid synthesis. In addition, histological sections of rat testis will be examined to quantitate and characterize the Leydig cell population in old and young rats before and after stimulation.

Publications:

Harman, S. M., Danner, R. L., and Roth, G. S. Testosterone secretion in the rat: response to chorionic gonadotrophin delayed rather than diminished with age. Endocrinology (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00014-07 CPB								
PERIOD COVERED July 1, 1976 to September 30, 1977										
TITLE OF PROJECT (80 characters or less)  The Biochemistry of renin and renin substrate.										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT										
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">H. J. Chou</td> <td style="width: 33%;">Visiting Fellow</td> <td style="width: 33%;">CPB NIA</td> </tr> <tr> <td>OTHER:</td> <td>R. I. Gregerman</td> <td>Chief, Endocrinology Section</td> <td>CPB NIA</td> </tr> </table>			PI:	H. J. Chou	Visiting Fellow	CPB NIA	OTHER:	R. I. Gregerman	Chief, Endocrinology Section	CPB NIA
PI:	H. J. Chou	Visiting Fellow	CPB NIA							
OTHER:	R. I. Gregerman	Chief, Endocrinology Section	CPB NIA							
COOPERATING UNITS (if any)  J. H. Shaper, Department of Oncology, Johns Hopkins University Hospital										
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch										
SECTION Endocrinology Section										
INSTITUTE AND LOCATION NIA, GRC, Baltimore, Maryland 21224										
TOTAL MANYEARS: 1.4	PROFESSIONAL: 1.3	OTHER: 0.1								
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SUMMARY OF WORK (200 words or less - underline keywords)  This project explores the biochemistry of <u>renin</u> and <u>renin-substrate</u> , proteins involved in hypertension. The substrate is being purified and labeled in order to develop a new assay for renin. Other studies include chemical modification of the substrate and synthesis of <u>polymeric substrates</u> and <u>renin inhibitors</u> .										

## Project Description:

Objectives: The secretion of renin, an acid proteinase produced by the juxtaglomerular cells of the kidney, is under the control of a variety of factors. In the circulation the enzyme acts on a plasma glycoprotein to release angiotensin I, a decapeptide, which is then activated by C-terminal cleavage to a smaller octapeptide, angiotensin II, in the lung and other tissues. Angiotensin II in turn influences the secretion of aldosterone, principal mineralocorticoid of the adrenal, and has other direct effects on the cardiovascular and central nervous system. In certain pathologic conditions renin has a direct role in the pathogenesis of hypertensive disease, as it does in renovascular stenosis. Renin is also indirectly implicated in other forms of hypertension. The secretion of renin (and aldosterone) is markedly influenced by aging in man, and hypertension is an age-dependent disease.

Because of these latter considerations, our laboratory has had an interest in renin and angiotensin, especially in development of new techniques for measurement of the enzyme and its peptide products, in the biochemistry of the enzyme and its substrate, and the relevance of the renin-angiotensin-aldosterone system to normal and pathologic aging.

Methods Employed: Renin has been assayed by our previously published polymeric substrate assay. Renin substrate has been partially purified from porcine plasma by column chromatography on DEAE cellulose, Con A-Sepharose, and Sephadex G-100. Other techniques used are standard analytical procedures of peptide chemistry (amino acid analysis, high voltage electrophoresis, etc.). Localization of substrate is made by radioimmunoassay of angiotensin I generated with added renin.

Major Findings: I. Separation of Human Renal Renin and Pseudorenin by Affinity Chromatography on Hemoglobin-Sepharose-2B. Human pseudorenin resembles renin in its ability to form angiotensin I (AI) from the synthetic tetradecapeptide renin substrate (TRS) or from purified hog protein renin substrate (PRS). However, unlike renin, pseudorenin does not attack PRS nor does it produce AI from PRS in the presence of plasma, presumably due to the presence of pseudorenin inhibitors. In contrast to renin, which is primarily or exclusively produced in the kidney, pseudorenin has been found in 13 different tissues and in plasma. The separation of those two enzymes is complicated by the existence of multiple forms of renin and pseudorenin in human kidney tissue. However, both pseudorenin-free human renal renin and hog renin have been isolated by multiple step, ion-exchange and gel filtration procedures. To date, there have been no reports on the separation of these two closely related enzymes by a simple procedure. Because of the large amounts of pseudorenin activity in plasma and tissue extracts, this enzyme can interfere with assay of renin, even at pH 7.5. Consequently, only pseudorenin-free samples can be assayed for renin with confidence.

We have shown previously that generalized proteolytic activity ( $^{14}\text{C}$ -

glycinated hemoglobin substrate) present in crude human renal renin is not due to renin but to the presence of contaminating acid proteases. Although renin belongs to the group of "acid" proteases, it appears not to be a general protease. Using this consideration we have shown that human renal renin and pseudorenin activities can be easily separated in a single step by affinity chromatography on hemoglobin-Sepharose-2B. Renin and pseudorenin activities were monitored by their activities against the synthetic polymer substrate developed in this laboratory and PRS at different pH's. Under the conditions employed (0.1M sodium acetate, 1M sodium chloride, pH 3.5, 4°) renin does not bind to the affinity absorbent. However, pseudorenin is effectively bound to the absorbent and can be eluted after raising the pH to 6.5. Pseudorenin-free renin prepared by this method is free of proteolytic activity toward hemoglobin. This approach described here affords a rapid means of preparing pseudorenin free human renin and should be useful in future efforts to isolate this enzyme. This work has been presented at the annual meeting of the American Federation of Biological Chemists.

II. Polymeric Inhibitor of Renin: Pepstatin is a potent specific inhibitor of several acid proteases (pepsin, Cathepsin D, renin). The effect of pepstatin on the production of angiotensin I by inhibition of renin both in vivo and in vitro has been described. These results suggest that pepstatin might be useful in evaluating the role of the renin-angiotensin system under various physiologic and pathologic conditions. Pepstatin could also be of potential clinical usefulness in the differential diagnosis of hypertension and the detection of renovascular hypertension.

Experimental use of pepstatin has been precluded by the very low water solubility of the peptide and its short duration of action, apparently due to rapid clearance from the circulation. Synthetic polymers have been used as carriers for different drugs in order to prolong the duration of their activity. In order to circumvent both the problem of limitation of dosage due to solubility and duration of action, we have coupled pepstatin through its C-terminal carboxyl to several high molecular weight, water soluble polymers. Since the C-terminal carboxyl seems to be not essential for pepstatin's effectiveness, such conjugates were expected to maintain their potent inhibitory effects. The conjugates would presumably also exert in vivo effects of longer duration of action than pepstatin itself which would be expected to approach those of the survival time of the polymer in the circulation.

The most promising pepstatin-conjugate polymer we have prepared to date is one in which the peptide is coupled to high molecular weight dextran, a relatively non-allergic, soluble polysaccharide. An essential feature in the success of the synthesis is the use of low concentration of cyanogen bromide to avoid irreversible precipitation of the polysaccharide during the activation reaction. Coupling of pepstatin to ethylene diamine dextran conjugate is performed in a 1:1 mixture of pyridine and 0.1M sodium phosphate, pH 5.5, with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide as the coupling catalyst. In this solvent system all reactants are soluble. Removal of the unreacted component is accomplished by prolonged dialysis. After coupling

of pepstatin to dextran an excess of glycine is added to saturate unreacted imidocarbonate groups, thus preventing the initially soluble conjugate from becoming insoluble during freeze-drying of the preparation.

Amino acid analysis indicates that 1 mg of polymer conjugate contains 20 $\mu$ g of pepstatin. Control experiments without added carbodiimide shows absence of pepstatin, indicating that the peptide does not become non-specifically adsorbed by dextran during preparation. The solubility of dextran-pepstatin conjugate is the same as that of uncoupled dextran. The polymeric conjugate maintains the same potent inhibitory effects as pepstatin itself toward human renin and porcine pepsin. The pepstatin conjugate also shows inhibitory effects on human renin using partially purified hog renin substrate or human renin substrate with determination of the angiotensin I product by radioimmunoassay. Evaluation of the effectiveness of the pepstatin conjugate in vivo is in progress. This work has been presented at the 5th American Peptide Symposium.

III. Chemical Nature of Renin Substrate. We have purified hog renin substrate to 75% purity (12  $\mu$ g of angiotensin I per 1 mg of protein) by ammonium sulfate precipitation, DEAE-Sephadex, Con A-Sepharose, and gel filtration. Hog renin substrate can be separated into five different forms on DEAE cellulose column with a pH gradient. We have attempted to separate these different forms on Con A-Sepharose column by eluting with different concentrations of  $\alpha$ -methyl glucoside or with a pH gradient but have obtained only one form. This result implies that the carbohydrate content of the different forms of hog renin substrate is probably similar not only in composition but also in chemical behavior. We also found that human renin substrate binds to wheat germ agglutinin which indicates that the reactivity of the carbohydrate in human renin substrate is similar to di-N-acetylchitobiose. Combination of Con A-Sepharose and wheat germ agglutinin-Sepharose chromatography should enable us to perform large-scale preparations of renin substrate.

It has long been known that hog renin does not hydrolyze human renin substrate. We have now found that hog renin does hydrolyze human renin substrate which was treated with either 10 mM dithioerythritol (DTT, Cleland's reagent). The data indicate that the conformation of human renin substrate is determined by an-s-s-bond and is an important factor in determining the specificity renin-renin substrate reaction. These studies are being prepared for publication.

IV. Labeling of Renin Substrate. We have prepared <sup>125</sup>I-Bolton-Hunter reagent and tested the material with a successful labeling of insulin. We have also labeled angiotensin I with this reagent. Separation of the reaction mixtures is in progress. Hopefully, we will have Bolton-Hunter derivatized angiotensin I as a carrier. We plan to label the hog renin substrate as soon as the carrier is ready. The suitability of this new radiolabeled protein renin substrate will then be examined.

Significance to Bio-medical Research and the Program of the Institute. Our studies have defined the chemical relationship of renin to other proteinases. Present work may allow an explanation of renin's specificity and the development of new classes of renin inhibitors and labeled protein renin substrates. This information may be useful eventually for practical applications to problems related to the diagnosis and treatment of hypertensive diseases.

Proposed Course of the Project. Our immediate objective is further preparation of protein renin substrate and its labeling. Once the labeled protein renin substrate has been made, kinetic studies will be undertaken.

Publications: None.

## PERIOD COVERED

July 1, 1976 to September 30, 1977

## TITLE OF PROJECT (80 characters or less)

The Baltimore Longitudinal Study of Human Aging

## NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Andres	Chief, Clinical Physiology Branch	CPB NIA
	A. H. Norris	Chief, Human Performance Section	CPB NIA
	N. W. Shock	Scientist Emeritus	NIA

OTHER: Other workers who are associated with the Longitudinal Study describe their involvement in their individual reports.

## COOPERATING UNITS (if any)

Baltimore City Hospitals

## LAB/BRANCH

Clinical Physiology Branch

## SECTION

Human Performance Section

## INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

## TOTAL MANYEARS:

8.55

## PROFESSIONAL:

2.10

## OTHER:

6.45

## CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS☒ (b) HUMAN TISSUES☐ (c) NEITHER☐ (a1) MINORS ☒ (a2) INTERVIEWS

## SUMMARY OF WORK (200 words or less - underline keywords)

The Baltimore Longitudinal Study serves as a resource for scientists working in the field of Gerontology. It provides a well-described group of men between 20 and 96 years of age for studies of the mechanisms of human aging. Projects in physiology, biochemistry, psychology, nutrition, pharmacology, endocrinology, sociology, and genetics, have been carried out or are in progress.

Objectives: The Baltimore Longitudinal Study provides a well described group of subjects as a resource in support of a wide variety of scientific investigations in gerontology and other disciplines. While long-term planning is encouraged, important studies of shorter duration have also been undertaken. The long-term general goals of the project are to: (1) secure replicate measures of physiological, pathological, biochemical and psychological variables on longitudinal study participants at specified intervals; (2) summarize and compare the results of testing in relation to age according to cross-sectional and longitudinal formats; (3) identify characteristics of individual participants which may be related to changes of function over time and to age at death; and, (4) determine whether the data obtained support one or another theory of the mechanisms responsible for age-related functional decrements.

Methods Employed: The Sample: Study participants are male volunteers recruited by other participants in the program. Recruits agree to return to GRC in Baltimore for 2-1/2 days of testing every 12 months (age 70 and over), 18 months (age 60-69) or 24 months (under age 60) for an indeterminate period. At entry into the program, 87% of subjects reported at least some college, 87% were identified with professional, technical or managerial occupations, 90% were presently married, 83% described themselves as financially comfortable or better, and of the group who returned for the fifth visit, 90% had rated their health as good or excellent on both first and fifth visits.

Data Management: Medical records and test results are maintained in written form in the laboratory and transferred to a data retrieval and analysis system by keypunching on tabulation cards or by recording the test results directly on punched paper tape or magnetic tape. Data are maintained and used in ways which protect the privacy of participants. Sensitive material is specially encoded. Individual scientists review, evaluate and summarize the data for scientific reporting.

Major Findings: This Project includes information about maintenance of the sample, and planning and operation of the overall study. Research findings are included in reports of investigators who use longitudinal study participants as subjects in their work. On June 30, 1977, 1087 participants had been tested during one or more visits to the GRC. There was a total of 6011 participant visits since 1958. Seven hundred and eighty-seven subjects had been tested 3 or more times, 607 had been tested 5 or more times, 327 at least 8 times, 169 at least 10 times and 70 had been tested 12 or more times. Since the beginning of the study, 168 participants have died and 265 have withdrawn from the study, leaving a total active sample of 654 men.

Several approaches have been made to the development of indices of what has been called functional age, physiological age and biological age as contrasted to chronological age. The reasons for and potential uses of these indices vary.



There is wide-spread dissatisfaction with the use of chronological age as an index of performance to be used to set the time of retirement. Many have agreed that functional capacities of each individual should be used to determine the time of retirement rather than chronological age alone. To this end many attempts have been made to establish an index of functional age. However, none of these attempts has been successful. The broad spectrum of tests administered and the long period of serial observations available from the Baltimore Longitudinal Study of Aging is a unique opportunity to develop an overall index of physiological performance and to test its validity. The literature has been surveyed and a study to develop an index of physiological age is being planned. The validity of the index will be determined by estimating the degree to which it changes in specific individuals over a 15-year period, a procedure which is only possible because of the accumulation of observations which have been made with the Baltimore Longitudinal Study. Furthermore if the index has validity, then it should correlate with certain deleterious end-points in the participants, such as the development of discrete disease states, institutionalization, and death.

Significance to Bio-Medical Research and the Program of the Institute:

A major goal of the longitudinal program is a deeper understanding of age-related changes in the different organ systems, and their interrelationships. The relation of functional changes in an individual to age at death, age of onset of a disease, and other end points is important for understanding aging in humans and the impact of aging on society. The intensive study of multiple variables will also provide tests of risk-factor theories for specific age-related diseases.

Proposed Course: Data collection and analyses will be continued. Continued emphasis on automation of tests, data entry, and analyses should provide improved accuracy and efficiency. A major summary of all aspects of this program is in progress.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00016-22 CPB									
PERIOD COVERED July 1, 1976 to September 30, 1977											
TITLE OF PROJECT (80 characters or less)  Age Changes in Human Performance											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT											
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: A. H. Norris</td> <td style="width: 33%;">Chief, Human Performance Section</td> <td style="width: 33%;">CPB NIA</td> </tr> <tr> <td>S. P. Tzankoff</td> <td>Sr. Staff Fellow</td> <td>CPB NIA</td> </tr> <tr> <td>OTHER: N. W. Shock</td> <td>Scientist Emeritus</td> <td>NIA</td> </tr> </table>			PI: A. H. Norris	Chief, Human Performance Section	CPB NIA	S. P. Tzankoff	Sr. Staff Fellow	CPB NIA	OTHER: N. W. Shock	Scientist Emeritus	NIA
PI: A. H. Norris	Chief, Human Performance Section	CPB NIA									
S. P. Tzankoff	Sr. Staff Fellow	CPB NIA									
OTHER: N. W. Shock	Scientist Emeritus	NIA									
COOPERATING UNITS (if any) Baltimore City Hospitals A.T. Welford, Dept. of Psychology, University of Adelaide, South Australia R. Fitzgerald, The Johns Hopkins Medical Institutions, Baltimore											
LAB/BRANCH Clinical Physiology Branch											
SECTION Human Performance Section											
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224											
TOTAL MANYEARS: 2.95	PROFESSIONAL: .90	OTHER: 2.05									
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords)  The purpose of this project is to study the mechanisms and the limitations of a variety of physical activities in old and young individuals. <u>Muscular activity</u> ranges from brisk walking on an inclined <u>treadmill</u> to <u>tapping</u> between targets of various widths drawn on paper and separated by various distances. <u>Exercise responses</u> are measured for <u>blood pressure</u> , <u>heart rate</u> , <u>pulmonary ventilation</u> , <u>carbon dioxide elimination</u> , and <u>oxygen uptake</u> . The oxygen cost of exercise is measured and compared to the total amount of <u>physical work</u> performed to estimate the <u>mechanical efficiency</u> of the subjects' <u>neuromuscular</u> and <u>psychomotor control systems</u> . Responses of the pulmonary system are interpreted in terms of standard <u>spirometry</u> and dead space ( <u>residual volume</u> ) measurements as well as studies of <u>respiratory control</u> . Limitations on performance imposed by cerebrovascular, cardiovascular, and pulmonary disease is assessed. <u>Reflex time</u> , <u>reaction time</u> and speed and accuracy of movement are measured and compared with exercise responses.											

Objectives: This project is designed to study the effects of aging on the physiological responses to and recovery from exercise--to describe age changes and to elucidate the mechanisms of these effects of aging. It is designed to identify underlying factors in the limitation of work performance and reduced mechanical efficiency in older people. For this purpose, detailed evaluation of pulmonary function and pulmonary response to stressful agents are carried out. Other factors such as the metabolic cost of limb movement and psychomotor control of limb movement are being studied.

An additional goal is to identify and explain the role of disease-altered physiological function in age-related limitation of work performance. Cerebrovascular, cardiovascular and pulmonary disease and functional measures such as blood pressure, reflex time, and reaction time will be considered.

Methods Employed: Measured amounts of physical work are administered to subjects of varying ages by means of a calibrated arm ergometer and quantitative mechanical analysis of limb movement. A treadmill is used to induce higher levels of work. Measurements of oxygen uptake,  $\text{CO}_2$  elimination, pulmonary ventilation volume, heart rate, blood pressure, and electrocardiogram are made before, during and after standardized amounts of exercise. The functional capacities of the pulmonary system are evaluated. Alterations in respiratory function as a result of the stimulation of low oxygen and high oxygen and carbon dioxide in the inspired air are evaluated by pressure changes induced by occlusion of airflow ( $P_{O_1}$ ).

Subjects of the BLS continue to be evaluated for participating in the multi-purpose maximal treadmill exercise tests. Subjects are instructed to walk on the motor-driven treadmill at a constant speed of 5.6 km/hr. They start on the level and the grade is elevated by 3% increments every two minutes until either exhaustion or, at the discretion of the attending physician, the test is terminated. Before the walk, as well as during the exercise and recovery from it, electrocardiographic tracings are displayed and monitored on a CRT, recorded on magnetic tape, and periodic samples reproduced on strip-chart paper. In addition while walking, subjects breathe through a mouthpiece-valve arrangement which allows for the inspiration of room air and expiration into spirometers for measurement of pulmonary ventilation, and after gas analyses, for the calculation of oxygen consumption, carbon dioxide production, and the respiratory exchange ratio for each level of exercise.

In the recovery phase venous blood samples are obtained at 3, 5, and 7 min. for the determination of lactic acid concentration, a by-product of anaerobic metabolism. Each subject who progresses through the test until exhaustion is asked to identify his limiting symptom, e.g., muscle pain, shortness of breath, general fatigue, and this response recorded. In addition, the investigator makes a subjective evaluation regarding whether or not the

performance represented a maximal effort. Exercise and recovery electrocardiographic tracings are evaluated for signs of ischemic coronary heart disease (IChD) according to the World Health Organization standards.

Major Findings: The data base on metabolic, pulmonary, and cardiovascular responses to maximal and submaximal exercise has been expanded from 56 to 201 participants in the past year. All were negative for IChD. These measurements will be repeated on future visits if the subjects meet the strict medical criteria. Mean values of maximal oxygen consumption were lower for each succeeding age-decade group. The values for those in their 60's were 27% lower than for those in their 20's. In a subsample for whom data on muscle mass were available, preliminary calculations indicated that the 27% difference between young and old was reduced to only 9% if oxygen consumption was related to muscle mass. This suggests that in these healthy men the principal cause for a reduced maximal work capacity is the loss of muscle tissue.

In performing submaximal work at 3, 6, and 9% grade, there were no significant differences in heart rate or oxygen consumption per kg body weight across these five age-decades (20's to 60's). This indicates no difference in the efficiency of performing light to moderate work. It does, however, underscore the reality that at any given work load, the older men must use greater fractions of their capacities to sustain the work.

Blood lactic acid concentrations at 3, 5, and 7 min of recovery from maximal exercise are now available for each of 154 subjects. As before, there are marked decreases with age (34% decrease between those in their 20's and those in their 60's) in the maximal values attained. The multiple samples through recovery show that there may be an age-dependent increase in the time required for lactic acid to diffuse from the muscles into the circulation. This delay must be accounted for when the measurement for maximal blood lactic acid is performed. The present data indicate that a single blood sample taken at 6 min of recovery will, on the average, represent about 98% of the maximal concentration in men aged 20 to 70 years. The literature, especially poor for data on older men, suggests a time between 2 and 5 min of recovery.

Preliminary data analyses indicate that there is a reduction, and eventual abolition, of the exercise-induced growth hormone secretion with age. Plasma samples obtained at 3 and 7 min of recovery from maximal exercise on 63 men (14 each in the 3 decades 20 to 49 yr and 7 each in the 3 decades 50 to 81 yr) were assayed for growth hormone concentration. Mean values for growth hormone concentration were 8.5, 5.3, 3.2, 3.2, 1.6, and 1.9 ng/ml, respectively, for each of the 6 age-decades. There were no age differences in the basal levels which ranged from 1.5 to 2.0 ng/ml.

The physiological significance of growth hormone in adults is poorly understood. Because growth hormone has been shown to stimulate skeletal muscle protein replication, its decreased secretion with age in response to maximal exercise may have significance in terms of skeletal muscle maintenance.

Significance to Bio-Medical Research and the Program of the Institute:

The decline of the ability of some older people to perform their day-to-day activities and to engage in pursuits which contribute to the economic and social strength of our society represents a national loss. Identification of the physiological, medical and social correlates of high levels of physical strength and psycho-motor performance in middle and old age, as well as declines in these abilities, should lead to techniques designed to reduce the rate of decline in performance capacities with age.

Proposed Course: Measurements of muscle strength and maximum power generating ability during arm exercise will be continued. Cardiovascular, ventilatory and metabolic responses to standardized arm ergometer exercise and monitored treadmill exercise will be used to classify participants into fitness categories and to explore the age relationships of biochemical and metabolic responses to exercise. Measurements of lung volumes and uniformity of pulmonary ventilation will be made to characterize the respiratory competence of the longitudinal studies participants. Measurement of respiratory drive in relation to various stimuli will be evaluated in these participants.

Publications:

Fish, J. E., Rosenthal, R. R., Summer, W. R., Menkes, H., Norman, P. S., and Permutt, S.: The effect of atropine on acute antigen-mediated airway constriction in subjects with allergic asthma. Am. Rev. Respir. Dis. 115: 371-379, 1977.

Fitzgerald, R.S., Garfinkel, F., Silbergeld, E., and Loscutoff, S.: Factors in the interpretation of mouth occlusion pressure during measurements of chemosensitivity. Chest. 70: 145-149, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00017-19 CPB
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PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Age Relationships of Body Composition, Nutrition and Physical Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.H. Norris	Chief, Human Performance Section	CPB NIA
	S.P. Tzankoff	Sr. Staff Fellow	CPB NIA
OTHER:	R. Andres	Chief, Clinical Physiology Branch	CPB NIA
	N.W. Shock	Scientist Emeritus	NIA
	C.H. Barrows	Acting Chief, Lab. of Cellular & Comparative Physiology	LCCP NIA
	R. Aamodt	Chief, Whole Body Counter Section	NM CC

COOPERATING UNITS (if any)

P.T. Davis, Dept. of Medicine, University of Buffalo, Buffalo, N.Y.

G. Borkan, S.M. Garn, Ctr. for Human Growth & Development, University of Michigan  
Ann Arbor, Michigan

LAB/BRANCH

Clinical Physiology Branch

SECTION

Human Performance Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:

2.95

PROFESSIONAL:

1.05

OTHER:

1.90

CHECK APPROPRIATE COX(ES)

☒ (a) HUMAN SUBJECTS

☒ (b) HUMAN TISSUES

☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study of the interrelationships of body composition, nutrition and physical activity is a longitudinal study of aging. It provides a description of these characteristics for participants in the Baltimore Longitudinal Study. It provides opportunity to relate changes in these basic characteristics of the individual participants to changes in other biochemical, physiological and psychological measurements. A variety of non-invasive techniques are employed. They include the Behnke Anthropometric Index, skinfold thickness measurements, height, weight, twenty-four hour creatinine excretion, total body potassium determination, basal metabolism determinations, Garn X-ray fat thickness measurements, a diet diary, and an activity questionnaire. Previously, measures of total body density and total body water have been made in longitudinal studies participants. Body density corrected for differences in body water content have been compared with the Behnke Index and other conventional anthropometric indices (such as ponderal index).

Objectives: This project is designed to describe age differences and age changes in body composition, nutrition, and physical activity. Mechanisms of interaction of these functions and behaviors will be sought. The relationship of these measurements to other physiological, psychological and biochemical variables will be examined.

Methods Employed: Height, weight, and body circumferences of longitudinal study participants are obtained by standard anthropometric methods. Roentgenographic and anthropometric estimates of skeletal mass are combined with height, weight, and body circumferences to provide an estimate of body fat. Other estimates of fat include skinfold thickness measurements and fat thickness measurements from X-rays. Indices of lean body mass include: (1) basal metabolic rate determinations, (2) twenty-four hour urinary excretion of creatinine, (3) total body potassium, and (4) total body water and extracellular water determinations by indicator dilution. Nutrient intakes and activity calories are estimated from a diary and a self-administered questionnaire. All such measurements are repeated in the course of each subject's participation in the longitudinal program.

Major Findings: Longitudinal analyses of the relation between the Basal Metabolic Rate (BMR) and the 24-hr Creatinine Excretion (CREX) have been completed. The BMR, as estimated from the rate of oxygen consumed per unit time is a reproducible measure of the rate of energy transformation consistent with the maintenance of body function. Creatinine is a by-product of skeletal muscle metabolism and is excreted unchanged in the urine. In the absence of gross renal impairment and/or muscle pathology, the amount of creatinine excreted over a period of time, e.g., 24 hours, is proportional to the mass of muscle which produced it. As recently shown in this laboratory, the relation between BMR and CREX affords the separation of the whole-body BMR into two energy-transforming components, one comprised of muscle alone and the other of the aggregate of other organs.

BMR values have long been known to decrease with age. Last year we reported that in cross-sectional analysis the progressively lower values with age of whole-body BMR were attributable to lower skeletal muscle masses. The rate of oxygen consumption by non-muscle tissues was similar among all age-decade groups.

To test the hypothesis that loss of muscle mass is characteristic of the aging process, data on 355 participants of the Baltimore Longitudinal Study with five or more paired values for BMR and CREX were examined for longitudinal changes. Subjects with diagnosed disorders involving thyroid, glucocorticoid, or sex steroid therapy were not included in the analyses.

Subjects were distributed into age-decade groups according to their mean ages over the years under observation. Each subject's contribution was the best-fit least-squares linear regression line for his data. For each age-decade group, all individual lines contributed to an average line centered on the mean age for that group. The slope of the line represented the mean change over the average years of observation.

Examination of the longitudinal data for whole-body BMR revealed no serious discrepancy from what the cross-sectional analyses had suggested. Each increasing age-decade group had successively lower mean values for BMR. Within each group, the negative slopes indicated progressive declines in BMR in the direction of the next older age-decade group.

Of the 355 men included in the analyses, 48 had died. In each age-decade group for the dead there was a consistent increase in the rate of non-muscle oxygen consumption compared with no change for the age-matched group of living. The slopes of these changes were similar in all age groups, both among the living and among the dead. Thus, when averaged overall, the mean rate of increase in oxygen consumption (ml/min/year) by non-muscle tissues was  $1.88 \pm 0.46$  for the dead and  $0.29 \pm 0.19$  for the living. Statistically, these differences are highly significant ( $t = 3.2$ ,  $p < 0.01$ ).

These observations allow for the following conclusions: 1) Oxygen consumption decreases consistently through adult age with men aged 65 and over exhibiting slightly faster declines; 2) The net decrease in BMR is wholly attributable to loss in skeletal muscle; 3) Subjects who died exhibited a lower rate of decrease in BMR through their terminal years which is attributable primarily to rise in the rate of oxygen consumption by non-muscle organs.

Undoubtedly, a number of the subjects now among the living will succumb over the next few years. For those who continue to participate in the BLS, there will be more longitudinal data on which a better compartmental analysis of oxygen consumption will be possible. It will be useful to identify a time of inflection, that is, the onset of rise in the non-muscle energy requirement so that it may be compared with other potential predictors of death.

The results of a collaboration that began in 1958 with Dr. Stanley M. Garn have been reported by Gary A. Borkan et al. Soft tissue radiographs were used to demonstrate the role of subcutaneous fat in altering external body dimensions in adult male participants in the Baltimore Longitudinal Study who were between 25 and 84 years of age. Both cross-sectional and longitudinal trends were analyzed. Weight of fat in the cross-sectional sample was found to be relatively constant with age while fat-free weight declined markedly after age 65. Longitudinal results indicated an increase in weight of fat for all age decade groups less than age 65 and a decline in the two age decade groups 65 and above. There were longitudinal declines in fat free weight for all age-decade groups except the 75-84 year group in which there was an increase. Longitudinal and cross-sectional trends for subcutaneous fat measurement were in agreement. In the trunk, subcutaneous fat increased in the region of the greater trochanter but decreased in the abdominal region through middle age. Abdominal diameter increased during this period, indicating enlargement or sagging of the abdominal contents. In the extremities, diameter of the calf and arm declined while fat was relatively stable, indicating a loss of lean tissue with age. This study agrees generally with earlier findings that age changes in body dimensions leading to thin extremities and thicker trunks are only partly attributable to fat redistribution.



An analysis has begun of the differences in nutrient intakes, body weight, and physical activity in 40 men who have died and 125 men who remain alive. These 165 subjects were age 65 and over and had at least 4 examinations here. Nutritional intake data were obtained from one-week dietary diaries and physical activity levels from a detailed questionnaire. Variables being analyzed are: Body weight and estimates of obesity; total caloric expenditure in physical activity; total caloric intake and intake of carbohydrate (simple and complex), protein, and fat (including class of fatty acid); vitamin, mineral, alcohol, and fiber intake. The programs are completed for analysis of differences between the dead and alive groups with respect to the levels of the listed variables and the rates of change in these variables during the study period of about 6 to 7 years.

#### Significance to Bio-Medical Research and the Program of the Institute:

Nutritional deficiencies in the aged are known to be common and are generally attributed more to the socio-economic deprivation of this group than to biological or physiological aging effects. The volunteers in the Longitudinal Study Group are not a deprived group--it may be characterized as upper-middle class and has a very high educational level. It, therefore, offers a unique opportunity to study nutritional status under very favorable conditions. The nutritional effects of biological age per se may, therefore, be separated from what might be called "social aging."

Certain age changes in organ systems and various diseases are thought to be affected by diet, level of physical activity, and body composition. From the repeated assessment of these factors over time, it may be possible to determine their relative contributions to longevity and the maintenance of health and vigor in later life. Difficulties associated with obtaining retrospective estimates of eating habits, activity and body composition in the past make a prospective approach necessary for the collection of reliable information.

Proposed Course: Studies of diet, physical activity and body composition will continue. Data already collected will be further analyzed. Interactions of changes in body composition food intake, food composition, kind and amount of physical activity, disease, and age will be examined. Specifically, body fat and lean body mass estimates, nutrient intakes and physical activity category will be used in an analysis of risk of cardiovascular disease and of rate of aging of several organ systems.

#### Publications:

Rose, C. S., Gyorgy, P., Butler, M., Andres, R., Norris, A. H., Shock, N. W., Tobin, J., Brin, M., and Spiegel, H.: Age differences in vitamin B6 status of 617 men. The Am. J. of Clin. Nutrition 29: 847-853, 1976.

Borkan, G. A. and Norris, A. H.: Fat redistribution and the changing body dimensions of the adult male. Human Biology (in press).

Tzankoff, S. P. and Norris, A. H.: The effect of muscle mass decrease on age-related BMR changes. J. Appl. Physiol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00018-11 CPB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Marital, Sexual and Social Factors in Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C. Martin  
OTHER: S.M. Harman

Sociologist  
Medical Officer

CPB NIA  
CPB NIA

COOPERATING UNITS (if any)

Baltimore City Hospitals  
H. Seideman, University of Maryland Baltimore Campus

LAB/BRANCH

Clinical Physiology Branch

SECTION

Human Performance Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:

1.15

PROFESSIONAL:

1.00

OTHER:

.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Systematic data concerning sexual, marital and social experience were obtained by structured interviews with males taking part in the Baltimore Longitudinal Study of Aging. In analysis 188 subjects aged 60-79 and married at interview were divided into thirds according to quantity of sexual activity reported for the preceding year. Subjective and behavioral variables were then related to subjects classified as least active, moderately active and most active sexually in order to identify factors affecting level of sexual functioning at these ages. Major correlates of being sexually most active were: a strong commitment to religious values, early farm residence, high socio-economic status, more abundant sexual activity during the earlier years of life, continued erotic responsiveness to visual stimuli, and unimpaired potency. Future plans are to relate variables derived from these interviews to such diagnostic entities as coronary artery disease, hyperlipidemia and other disabilities.

Objectives: Present goals are to: (1) learn whether individual differences in sexual functioning may be related to physiological, psychological or behavioral characteristics beyond those already considered, (2) determine whether coronary artery disease and other pathologies of unknown etiology may be associated with any attribute of prior marital, sexual or social experience in longitudinal subjects, (3) continue to interview subjects newly admitted to the longitudinal study, and (4) in collaboration with Dr. S. M. Harman's study of gonadotrophin secretion, pituitary gonadotrophin reserve and Leydig cell reserve, to update interviews to take into account changes in marital and sexual experience (Dr. S.M. Harman's identification number is Z01 AG 00023-02 CPB).

Methods Employed: The present investigator has now interviewed the majority of study members regarding their experience of marriage and sexual activity. In requesting interviews, the investigator describes study objectives in considerable detail, provides assurance of confidence and emphasizes the voluntary nature of such a contribution. Thus far, 16 subjects have declined the interview while 696 have completed interviews.

To aid fluency of communication and to insure the accumulation of systematic information, questions were memorized by the investigator in addition to whatever codes were deemed necessary for the classification of responses. With a view to rapport and the generation of data not obtained elsewhere in testing schedule, various aspects of residential, occupational, educational, religious, military and parental-home experience were reviewed before turning to questions concerning sexual conduct and marital adjustment. The data obtained are unique and are thought to be of excellent quality because of the high occupational and educational attainment of subjects and their evident interest in this aspect of the study.

Major Findings: Students of human sexuality have long been mystified by the observation that many males desire frequent sexual activity while others of like age are content with infrequent activity. Equally perplexing has been the fact that some older males are sexually vigorous whereas others become sexually unresponsive or encounter problems with potency. The availability of present data suggested relating a variety of experiential variables to current sexual functioning in order to identify correlates of sexual vigor.

Analysis was confined to 188 respondents aged 60-79 at interview who had lived with a spouse the full year prior to report. Subjects were stratified by age into five-year groups before each was subdivided into thirds according to number of sexual events reported for the preceding year. The procedure well-differentiated subjects by quantity of activity, with the least active group averaging 3.8 sexual events for the year, the moderately active group 20.0 sexual events, and the most active 62.3 events. The procedure also controlled for age at interview (mean 68.2 years) and year at birth (1900).

Respondents falling into the above categories were initially compared with regard to social attributes. Interestingly enough, factors found related to being most active were: a strong commitment to religious values, farm residence before 20 years of age, post graduate training, and professional and technical occupational status. Although the significance of these particular attributes for sexual functioning remains obscure, their appearance as correlates is reassuring since respondents with these attributes tend to hold conservative social and sexual attitudes and were not likely to deliberately overstate quantity of sexual experience.

Numerous variables proved independent of current levels of sexual functioning. These include such behavior as: age at marriage, number of years married before age 60, age at first coitus and number of coital partners before age 40. Most respondents were of the opinion that regular sexual activity is likely to be important for good health (69%), most stated they would not seek treatment to obtain greater sexual vigor, even if this were possible (62%), and most rated their current marital situation as highly successful (86%). However, none of these attitudes or evaluations were significantly related to current level of sexual activity.

On the other hand, large differences were found between least active and most active respondents pertaining to two areas of inquiry. The first finding concerned estimates of past levels of sexual activity. Specifically, most active subjects reported a significantly higher maximum number of coital events in any week of marriage, a higher customary frequency of coitus in early marriage, and greater abundance of sexual activity from 20 to 39 and from 40 to 59 years of age than were reported by the least active group. This finding, based on retrospective evidence, strongly suggested that the frequency of sexual activity at time of interview is reflective of individual differences in sexual vigor which had persisted over many years of the life history. This evidence of considerable continuity of sexual functioning over time is consistent with the observations of Pfeiffer and Davis, and of Masters and Johnson to this effect. These data are, moreover, of interest for they specify those individuals whose level of sexual functioning appears both minimally and maximally affected by the aging process.

Marked differences also were found between least active and most active subjects concerning responses to questions with respect to erotic reactions from seeing women and with respect to length of time comfortable without sexual activity. Roughly 3 out of 5 least active respondents indicated that they no longer recognized erotic arousal from these kinds of stimuli and were able to comfortably ignore sex for many months at a time or 'indefinitely'. In contrast, most respondents classified as most active remained cognizant of erotic reactions from visual stimuli and most cited intervals of less than a month as the time they could comfortably abstain from sexual activity.

Significance to Bio-Medical Research and the Program of the Institute:  
Other than providing statistical support for the concept that individual differences in sexual vigor tend to persist over the male life span and ultimately influence sexual functioning in old age, present findings reveal

additional relationships between subjective and behavioral variables which have received little attention. The evidence obtained indicates for example that much of the sexual inactivity of the older male is simply due to sexual apathy (unresponsiveness to stimuli which previously had evoked erotic reactions) rather than to negative sexual attitudes as suggested by Rubin.

Finally, it was found that subjects who stated they were no longer sufficiently responsive or erectile to effect coitus, also insisted they felt no pressure to perform, were relatively undisturbed by their lack of competence and had never sought help for their condition. These observations point up the fact that impotence in conjunction with a definite sense of sexual need is usually acutely disturbing although erectile failure in the absence of a sense of need is merely 'benign'. Thus, there is another dimension to impotence that is seldom appreciated by investigators whose observations are limited to patients in clinical practice.

Proposed Course: With the help of H. Seideman, graduate student, an effort is in progress to compare subjects in the above described categories of sexual activity levels with respect to a variety of measures of cognitive and physiologic function in anticipation of finding differentials. The effort is frankly exploratory. Plans are also underway to compare subjects with a diagnosis of coronary artery disease and their controls in search of differences in sexual, marital and social experience. This analysis is expected to be the first of a similar series of studies involving: hypertension, hyperlipidemia, diabetes, stroke, arthritis and prostatism.

#### Publications:

Martin, C. E. Sexual Activity in the Aging Male. In: Money, J. and Masaph, H. (Eds.): Handbook on Sexology, New York, Elsevier/North Holland, 1977, pp. 813-824.

Giambra, L. M. and Martin, C. E. Sexual daydreams and quantitative aspects of sexual activity: Some relations for males across adulthood. Archives of Sexual Behavior (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00021-14 CPB
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PERIOD COVERED  
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)  
Dermatoglyphics in: 1. Populations 4. Families  
2. Medicine 5. Twins  
3. Aging 6. Methodology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.C. Plato	Geneticist	CPB NIA
OTHER:	D.C. Gajdusek	Chief, Lab. of Central Nervous System Studies	CNS NINCDS
	R. Garruto	Sr. Staff Associate	CNS NINCDS
	F.S. Steinberg	Head, Analytical Unit	OPA NICHD
	B.D. Bricker	Computer Specialist	CPB NIA

COOPERATING UNITS (if any)

See attached page.

LAB/BRANCH  
Clinical Physiology Branch

SECTION  
Human Performance Section

INSTITUTE AND LOCATION  
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: .35	PROFESSIONAL: .25	OTHER: .10
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CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keyword:)

This project represents a joint collaborative effort, involving the WHO and other national and international biological laboratories to coordinate the evaluation and interpretation of the available dermatoglyphic data. Specifically the objectives of this project are: 1) to study the distribution of dermatoglyphics among the various human populations (population dermatoglyphics); 2) to establish the dermatoglyphic frequencies in normal control samples (control dermatoglyphics); 3) to establish dermatoglyphic markers in various diseases (clinical dermatoglyphics); 4) to study the dermatoglyphics of the aged; 5) to study the genetics of dermatoglyphics; and, 6) to utilize dermatoglyphics as an added tool in twin diagnoses (twin dermatoglyphics).

Cooperating Units:

Z01 AG 00021-14 CPB

1. W. Wertelecki  
Department of Genetics  
University of South Alabama  
Mobile, Alabama
2. J. T. Schwartz  
Division of Hospitals & Clinics  
Bureau of Medical Services  
Health Service Administration  
USPHS, West Hyattsville, Md.
3. R. MacLennan  
International Agency for Research on Cancer  
WHO, Lyon France
4. M. Alpers  
Institute of Medical Research  
Goroka, New Guinea
5. C. Bartsocas  
Department of Pediatrics  
University of Athens  
Athens, Greece
6. A. D'Alessandro  
International Center for Medical Research  
Universidad Del Valle  
Calik, Colombia
7. M. T. Newman  
Department of Anthropology  
University of Washington  
Seattle, Washington
8. R. W. Hornabrook  
New Guinea Institute of Medical Research  
Wadestown, Wellington, New Zealand
9. Y. Ahuja  
Department of Genetics  
Osmania University  
Hyderabad, India
10. G. M. Flickinger  
Department of Biology  
Xavier University of Louisiana  
New Orleans, Louisiana

11. W. Pollitzer  
Department of Anatomy  
The University of North Carolina at Chapel Hill  
Chapel Hill, North Carolina
12. R. Chakraborty  
Center for Demographic and Population Genetics  
Graduate School of Biomedical Sciences at Houston  
The University of Texas at Houston  
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13. J. Larrick  
Duke Medical School  
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Durham, North Carolina
14. B. Schauman  
Neurology Section  
The Veterans Administration Hospital and  
The University of Minnesota  
Minneapolis, Minnesota
15. R. G. Schamschula  
The Institute of Dental Research  
The United Dental Hospital of Sydney  
Sydney, Australia

Project Description:

Objectives: This ongoing project represents an extensive collaborative effort, in conjunction with WHO and other national and international institutions, to study all aspects of dermatoglyphics. The specific objectives of this study are: (1) to establish the distribution of dermatoglyphic features in various populations, with special emphasis on primitive and other isolated groups in the South Pacific and other parts of the world. (2) To establish associations between dermatoglyphic features and specific clinical anomalies. (3) To study the dermatoglyphic frequencies in different age groups. (4) To investigate the genetic aspects of dermatoglyphics through family and twin data. (5) To improve and standardize the international methodology and nomenclature on dermatoglyphics.

The overall purpose of these studies is to utilize the dermatoglyphic markers in an effort to study the genetic structure of different populations, and to provide additional tools for studying the etiology of certain diseases.

Methods: Digital and palmar prints collected by different groups through various methods are sent to our laboratory for evaluation and interpretation of the results.



Major Findings: (1) Dermatoglyphics and Aging: The comparison of the dermatoglyphic frequencies of two different samples of adult Caucasians to those of a sample of seven year old normal controls showed that the older groups had narrower atd angles, higher frequencies of aberrant simian creases and more transverse palmar ridges. The young vs. adult comparisons indicate a trend (though not statistically significant) for lower frequencies of accessory triradii and patterns in the IV palmar interdigital areas. The dermatoglyphics of the Baltimore Longitudinal Study were separated into four age groups, 30-44, 45-59, 60-74 and 75+ years of age. Intragroup comparison gave significant differences in the presence of patterns in the hypothenar area and the presence of aberrant simian flexion creases. In addition to the Baltimore Longitudinal Study data, we also studied the dermatoglyphics of 350 seven year old males, collected from the participants of the Perinatal Research Study (NINCDS) in Boston. The comparisons of a group of seven year olds with each of the older age groups gave increasing number of dermatoglyphic differences with increasing age group. These results suggest selection process operating on dermatoglyphic traits which may be associated with diseases which terminate life prior to reaching the later years. It is also possible that the dermatoglyphic differences between the seven year old and adult samples may reflect a genetic heterogeneity of the populations (Boston and Baltimore) from which these samples were selected. We are presently collecting dermatoglyphic data of all age groups from the same population in order to retest and verify our findings.

(2) Dermatoglyphics and Twins: By comparing the dermatoglyphics of monozygotic twin, dizygotic twin and sib pairs we were able to show that most dermatoglyphic traits, although genetically controlled are influenced to a certain degree by the intrauterine environment.

(3) Population and Methodology Studies: We standardized most of the reported dermatoglyphic data and prepared (a) an exhaustive description of the dermatoglyphics of the North, South and Central American Indians and Eskimo, (b) an Atlas of the distribution of the most useful dermatoglyphic traits in six major human groups, Amerindians, Australasians, Negroes, Caucasians, Orientals, and Asian Indians. (c) We established the dermatoglyphic frequencies in several Australasian and South American isolates as part of the overall genetic and medical study and characterization of these groups.

Significance to Bio-Medical Research and the Program of the Institute:

1) To utilize dermatoglyphics as an added genetic marker in the overall study of aging. 2) To provide internationally standardized dermatoglyphic frequencies which could be used, as control data in the studies of disease associations and for facilitating factor analyses in evaluating the studies of genetic relationship among isolates.

Proposed Course: To continue this project by further evaluating the data at hand and by the collection of additional clinical and population dermatoglyphic data from subjects of all ages.

## Publications:

Plato, C.C.: DERMATOGLYPHICS: A review (in Greek). *Materia Medica Greca*, Special suppliment on Human Genetics. 4:246-257, 1976.

Plato, C.C. and W. Wertelecki: The dermatoglyphics of American caucasian adults: with a review of the caucasian dermatoglyphics. Proceedings of the Bartos Dermatoglyphics Symposium, Bratislava, Czechoslovakia, 121-134, 1977.

Ahuja, R., J.S. Murty, C.C. Plato and T.J. Schwartz: Inheritance studies of c-t and a-d intertriradial differences of the palm by means of twin pair analysis. Zeits. Morph. Anthropol. 68:220-225, 1977.

Plato, C.C.: Dermatoglyphics and Aging. J. of Geront. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF          INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER  Z01 AG 00022-01 CPB
PERIOD COVERED July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (90 characters or less)  Epidemiological Investigations of Osteoarthritis of the Hand		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	C.C. Plato A.H. Norris	Geneticist Chief, Human Performance Section
		CPB NIA CPB NIA
OTHER:	D.C. Gajdusek R.M. Garruto	Chief, Lab. of Central Nervous System Studies Sr. Staff Associate
		CNS NINCDS CNS NINCDS
COOPERATING UNITS (if any) I. Higgins Tecumseh Michigan Community Health Study The University of Michigan Ann Arbor, Michigan		
LAB/BRANCH Clinical Physiology Branch		
SECTION Human Performance Section		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.50	.35	.15
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  The overall objective of this project has been the <u>epidemiological study of osteoarthritis or degenerative joint disease of the hand</u> . Specifically, this project deals with: 1) the joint-digit prevalence of the disease; 2) its familial aspects; 3) its bilateral symmetry; 4) its association with selected physiological and anthropometric variables; 5) longitudinal changes in prevalence of the disease; and, 6) the prevalence of osteoarthritis among patients with Amyotrophic Lateral Sclerosis/Parkinsonism Dementia of Guam and among Guamanian controls.		

Objectives: The objectives of this investigation have been: 1) To study the epidemiological, familial and longitudinal aspects of osteoarthritis of the hands of participants of the Baltimore Longitudinal Study, by grading and evaluating each joint of each digit separately; 2) To study the prevalence of osteoarthritis in the thoracic spine of the same participants and to establish possible associations between the thoracic and hand joint degeneration; 3) By using the available x-rays of both hands from participants of the Tecumseh Michigan Community Study and by collecting bilateral hand x-rays from our own (GRC) participants to study possible differences in the prevalence of osteoarthritis in the left and right hands; 4) To study the prevalence of osteoarthritis among the patients with Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex of Guam and among the normal control Guamanians.

Methods Employed: Radiographs were graded separately for each of the proximal and distal interphalangeal joints utilizing the internationally accepted grading system of J. H. Kellgren. Grades 0, 1 were considered normal and grades 2, 3 or 4 were considered as having osteoarthritis (affected). With the exception of bilateral symmetry, all other studies involved x-rays obtained from the subjects of the longitudinal study of the Gerontology Research Center. The GRC data involved only left hand x-rays. For the bilateral symmetry study, we graded the x-ray films of both hands from one hundred male and one hundred female participants of the Tecumseh Study.

Major Findings: Our results suggest that the occurrence of osteoarthritis in the distal and proximal interphalangeal joints may be of different etiology. Degeneration of the distal interphalangeal joints is more prevalent and usually more severe than that of the proximal interphalangeal joints. Distal joint osteoarthritis is longitudinally related to the age of the individual while proximal joint osteoarthritis is not. Regardless of joint type, the right hands are more frequently and seriously affected than the left hands. Finally, regardless of type of joint or hand, the fifth and the second digits are most vulnerable to osteoarthritis.

Significance to Bio-Medical Research and the Program of the Institute:

The present study offers a new and more refined approach, joint-digit, to the evaluation of osteoarthritis of the joints of the hand, provides information on the bilateral and interdigital differences in the prevalence of osteoarthritis and contributes towards our efforts in defining the etiology of this disease.

Proposed Course: Two manuscripts have been submitted for publication and two others are in the manuscript preparation stage. The study will be followed up with the evaluation of the collected bilateral hand x-rays and the x-rays of the thoracic vertebral column. We will continue the collection of hand x-rays from the participants of the longitudinal study. We have already initiated the collection of bilateral hand x-rays from patients with Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex of Guam as well as from normal Guamanian controls.

Publications:

Plato, C. C.: Osteoarthritis of the hand: Epidemiological, longitudinal and familial studies. Doctoral dissertation. The University of Michigan, Ann Arbor, Michigan, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER  Z01 AG 00027-01 CPB									
PERIOD COVERED July 1, 1976 to September 30, 1977													
TITLE OF PROJECT (80 characters or less)  Functional Lateral Dominance and Bilateral Asymmetry													
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table> <tr> <td>PI:</td> <td>C.C. Plato</td> <td>Geneticist</td> <td>CPB NIA</td> </tr> <tr> <td>OTHER:</td> <td>A.H. Norris</td> <td>Chief, Human Performance Section</td> <td>CPB NIA</td> </tr> </table>						PI:	C.C. Plato	Geneticist	CPB NIA	OTHER:	A.H. Norris	Chief, Human Performance Section	CPB NIA
PI:	C.C. Plato	Geneticist	CPB NIA										
OTHER:	A.H. Norris	Chief, Human Performance Section	CPB NIA										
COOPERATING UNITS (if any)													
LAB/BRANCH Clinical Physiology Branch													
SECTION Human Performance Section													
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224													
TOTAL MANYEARS: .35		PROFESSIONAL: .25		OTHER: .10									
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS													
SUMMARY OF WORK (200 words or less - underline keywords)  The purpose of this investigation is to evaluate the various forms of <u>lateral functional dominance</u> , such as <u>hand, eye and foot preference</u> and to study their association with normal or <u>abnormal bilateral asymmetry</u> (osteoarthritis and bone loss) as well as with <u>normal bilateral asymmetry</u> seen in dermatoglyphics.													

**Objectives:** To study the various facets of lateral functional dominance and bilateral asymmetry. To investigate their possible interdependence or associations and their effect upon the health and longevity of an individual.

**Methods:** The sample is at present composed entirely of participants of the Baltimore Longitudinal Study. 1) Lateral Functional Dominance: The participants are asked to perform a number of simple tests designed to establish hand, foot and eye preference. 2) Bilateral Asymmetry: Collection of bilateral hand x-rays and dermatoglyphics. In addition to these data, we would be utilizing already collected data which demonstrate physiological functional or anatomical bilateral differences.

**Major Findings:** Preliminary findings showed that: 1) osteoarthritis is more prevalent and more severe in the right than the left hands of the same individual; 2) bone measurements from 290 sets (left and right) of x-rays of the hand indicated that the II metacarpal bone of the right hand is as a rule longer and wider than that of the left hand. The preliminary results are summarized below:

<u>Measurement</u>	<u>Paired Bilateral Comparisons (%)</u>		
	<u>L = R</u>	<u>L &gt; R</u>	<u>R &gt; L</u>
Medullary width	9.7	41.2	49.1
Overall width	8.3	28.3	63.4
Cortical Thickness	5.2	32.6	62.2
Total length	3.8	38.4	57.8

The cortical thickness (overall width-medullary width) indicates that the right metacarpal has higher bone mass than the left. This difference is brought about by the higher deposition of surface bone in the right rather than accelerated medullary bone absorption in the left metacarpal. The larger cortical thickness combined with the increased length makes the overall bone content of the right II metacarpal even higher than that of the left. The question arises as to whether this higher bone mass of the right II metacarpal is related to the well established higher frequency of right handed individuals. 3) Of the 272 participants tested for lateral functional dominance 5.9% were judged to be left handed, 2.2% ambidextrous, and 91.9% right handed. Preference for the left foot was 5.5% which is similar to that for handedness. Half of the left handed were also left footed. The 5.9% of left handedness in our sample is not enough to explain the results of bone mass. If higher bone mass was related to hand preference, we would expect the frequency of left handed in our sample to be between 25-35 percent. This is not the case even if we assume that most of the ambidextrous and some of the right handed were originally left handed trained to use the right. Preliminary estimates of the grip strength of the same sample showed that 12% had higher grip strength in the left hand and 15.4% had equal grip strength in both hands. These values are closer to the values of bilateral asymmetry in bone mass than the handedness values.

Left eye preference was 29.6%. Sinistral (left limb on top) arm folding, digital interlocking and foot overlapping were found to be 55.5%, 47.4%, and 40.8%, respectively. Only one of the 272 participants demonstrated the left side preference in all 13 tests. These data are presently being analysed further in detail in order to evaluate the overall picture of lateral functional dominance and its association with physiological and anatomical bilateral asymmetry.

Significance to Biomedical Research and the Program of the Institute:

These studies are intended to offer a deeper understanding of etiology, the epidemiology and genetics of lateral functional dominance and its possible role in the etiology of bilateral asymmetry either normal, as in dermatoglyphics, or abnormal as in osteoarthritis.

Proposes Course: In dermatoglyphics bilateral asymmetry is the rule rather than the exception. These bilateral differences are very distinct and universally uniform. This holds true regardless of the existing overall interracial differences in the distribution of the dermatoglyphic traits. The bilateral asymmetry in dermatoglyphics has been shown to be related to a certain extent with handedness. We are collecting additional data to study this association.

Publications: None





Objectives: Our involvement in the multidisciplinary project has three objectives: 1) to identify the epidemiological variables which contribute to the very high incidence of ALS/PD on Guam; 2) to determine the extent of genetic involvement in the etiology of the disease; and 3) to study the distribution of several established genetic markers in the normal Guamanian (Chamorro) population to be used as controls in the comparisons with the patients.

Methods: Through the years we have employed two methods. 1) Patient-Control registry panels (Registries) and 2) the collection of extensive family and whole village pedigrees. We initiated both of these programs almost twenty years ago and have updated them several times since that time. The patient-control registry panels include 136 ALS/PD patients, 136 individually matched controls and their respective first degree relatives (parents, sibs and offspring) and their spouses. The registries are periodically updated by recording all new cases among the registered relatives of both the patients and the controls. The individual pedigrees as well as the extensive pedigree of Umatac (the village with the highest prevalence of ALS/PD on Guam) are also periodically updated by adding not only the new cases but also marriages, new offspring and deaths.

Major Findings: Amyotrophic Lateral Sclerosis is about a hundred times higher on Guam than any other part of the world. The preliminary analyses of the patient-control registry panels indicated that the disease is familial, with significantly higher prevalence among the first degree relatives (mainly sibs) of the patients than the controls'. The preliminary segregation analysis of the Umatac pedigrees suggested that ALS/PD has a genetic etiology, possibly transmitted through a dominant gene with full penetrance in the male and 50% penetrance in the female.

Significance to Bio-Medical Research and the Program of the Institute: The ultimate goal of this multidisciplinary program is not only to elucidate the etiology of Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex of Guam, but also to provide a model for studying other neurological diseases and dementias which are for the most part diseases of old age.

Proposed Course: To reevaluate the last updating (February 1977) of the patient-control registries, pedigrees and other epidemiologic data and prepare manuscripts for publication.

Publications: None

ANNUAL REPORT OF THE LABORATORY OF MOLECULAR AGING  
NATIONAL INSTITUTE ON AGING

The Laboratory of Molecular Aging conducts biochemical and biophysical research needed to: (A) determine the mechanisms by which physiological processes are altered in the aged; and (B) use this new information as a base for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities. Our research this year continues to focus on two critical areas known to undergo perturbations leading to the inability of the aged to maintain homeostasis, namely, physiological control and genetic information transfer systems. These investigations impact on the fundamental mechanisms of age-dependent changes in (1) renal function, (2) cardiac function, (3) cerebral function, (4) smooth muscle activity as it relates to hypertension, and urogenital and gastrointestinal function, (5) metabolism, and (6) on mechanisms basic to the development of aging hypothesis which transcend specific physiological systems.

A major locus of regulation in intermediary metabolism, and a problem of special concern in aging, is the interaction between the oxidation of carbohydrate and fat. This control is crucial, for during low levels of food intake and/or high work-loads the increased availability of fatty acids from reserve stores inhibits the oxidation of glucose in the heart and thus spares glucose for the brain, for which it is a mandatory fuel. The site of the regulation is the mitochondrial pyruvate dehydrogenase complex, which is inactivated during lipid oxidation, thus sparing pyruvate and, indirectly, glucose. Inactivation/activation mechanisms for control of the activity of the dehydrogenase complex was shown to be mediated by covalent-linked phosphorylation/dephosphorylation reactions. Heart pyruvate dehydrogenase was found to be sensitive to the ratios of intramitochondrial  $\text{NAD}^+/\text{NADH}$ ,  $\text{CoASH}/\text{acetylCoA}$ , and  $\text{ADP}/\text{ATP}$ , such that an increase in each ratio gave a decreased degree of pyruvate dehydrogenase phosphorylation, and an increase in enzymatic activity.

The effect of fatty acid oxidation was to enhance phosphorylation of the enzyme by the pyruvate dehydrogenase kinase, favoring the inactivated form of the enzyme. Mitochondria from hearts of aged (24 months) rats showed a significantly lesser effect of fatty acid oxidation on pyruvate dehydrogenase interconversion than did mitochondria from mature (6 months) animals. Thus, glucose was not spared. This finding suggests that the aged, when subject to specific types of stress, can not frugally reserve glucose for vital functions, e.g. brain metabolism.

The significantly lesser effect of fatty acid oxidation on pyruvate dehydrogenase interconversion could reflect either an alteration in the pyruvate dehydrogenase interconversion enzymes or a lesser activity of fatty acid oxidation in heart mitochondria from senescent animals. The latter possibility was investigated first. It was found that the rate of oxygen consumption was diminished by about 30% in mitochondria from the senescent animals ( $p < 0.005$ ) with palmitoyl-carnitine as substrate. This decrease was specific to fatty acid oxidation and was not seen with other NAD-linked substrates. It is not, therefore, a trivial artifact of mitochondrial preparation. In an attempt to define more

closely the locus of this age-linked decrement, the complex pathway of fatty acid oxidation to  $\text{CO}_2$  and water was dissected into several portions, by the use of appropriate substrates. In sequence, these portions are (1) carnitine: acylcarnitine exchange (entry of the acylcarnitine into the mitochondrion); (2) carnitine acyltransferase; (3)  $\beta$ -oxidation and (4) the tricarboxylate cycle. Whereas palmitoylcarnitine oxidation involves 1 through 4, acetyl-carnitine oxidation involves only 1, 2 and 4 whereas octanoate oxidation involves only 3 and 4, plus (uniquely) acyl-CoA synthetase. By use of these substrates and others, it was established that there are several different sites of decrement in activity with senescence.

Measurement of the activities of the individual enzymes suspected from the reasoning above to be decreased with senescence gave significant decreases in octanoyl-CoA synthetase, carnitine acetyltransferase and  $\beta$ -hydroxyacyl-CoA dehydrogenase. The similar degree of loss of activity (circa 30%) raises the possibility of a co-ordinated control of the synthesis (or degradation) of these enzymes.

The fact that the oxidation of both species of acylcarnitine which were tested was diminished with senescence and the normally rate-limiting character of mitochondrial transport processes, suggested that carnitine: acylcarnitine exchange across the mitochondrial membrane be measured directly. This was done and it was found that the process was less active in the mitochondria from senescent animals. However, further investigation revealed that this reflected a decreased pool of mitochondrial carnitine and an unchanged first-order rate constant of exchange rather than any change in the carrier molecule *per se*. In a very recent study a diminished whole-heart content of carnitine in senescence was reported, so that the mitochondrial preparations appear to give an accurate picture of the *in vivo* situation. These mitochondrial findings, *i.e.* the description of carnitine efflux from the mitochondria as a first-order process, the demonstration of an unchanged first-order rate constant and the demonstration of a diminished mitochondrial carnitine pool with senescence, all define a decreased rate of carnitine:acylcarnitine exchange in the senescent heart, with implications for all metabolic pathways involving that exchange.

An essential step in understanding the mechanisms by which heart muscle contractility changes with age is to define the transport processes which are basic to regulation of cardiac function. One such system, being investigated is the transport and intracellular compartmentation of  $\text{Ca}^{2+}$  by the sarcoplasmic reticulum. The duration of the active state in cardiac muscle is determined in part by the duration of the interval in which  $\text{Ca}^{2+}$  is bound to myofibrillar regulatory proteins; consequently, the rate of removal of  $\text{Ca}^{2+}$  by sarcoplasmic reticulum will influence the time course of muscle relaxation. Last year we reported that preparations of sarcoplasmic reticulum from hearts of old (24-25 months) rats showed significantly less uptake than membranes from hearts of young (6-8 months) animals. In completing these experiments it was found that the percent decline in transport activity in microsomes from aged hearts varied with  $\text{Ca}^{2+}$  concentration, decreasing from 57% at  $.33 \mu\text{M Ca}^{2+}$  to 24% at  $1.21 \mu\text{M Ca}^{2+}$ . The lower *in vitro* rates of  $\text{Ca}^{2+}$  accumulation in membrane vesicles from senescent animals suggest a possible biochemical mechanism to account for the observed increase in the time course of cardiac relaxation. In further studies

to define the relationship between active  $\text{Ca}^{2+}$  transport and mechanical function, rapid chemical quenching was used to compare the initial time course of phosphorylation in skeletal and cardiac muscle sarcoplasmic reticulum preparations. Both preparations exhibited a rapid initial phase of labelling which exceeded the steady state level by approximately 20%. The rates of decay of the phosphoprotein overshoot in both preparations were similar implying that the rates of dephosphorylation are similar. The presence of virtually identical phosphorylation kinetics in these preparations suggests that the slower rate of relaxation observed in cardiac muscle may be due to a lower density of transport sites rather than to a slower rate of turnover.

Digitalis is a therapeutic agent widely used in the treatment of heart failure in the elderly. It is known to increase the force of the cardiac muscle contraction and previous studies, by others, suggested that the inotropic response to the cardiac glycoside, ouabain, decreases with age. Ouabain acts biochemically by inhibiting membrane  $(\text{Na}^+ + \text{K}^+) \text{ATPase}$  activity. Therefore, the ouabain sensitivity of cardiac sarcolemmal  $(\text{Na}^+ + \text{K}^+) \text{-dependent ATPase}$  prepared from young and aged dogs was measured in conjunction with studies of the digitalis responsiveness of isolated muscle. Data obtained from 3 young and 3 aged dogs showed no significant difference in the dose response curve of the ATPase to ouabain. For both, the apparent inhibition constants  $\approx 3.5 \times 10^{-6} \text{ M}$ . Consequently, differences in muscle responsiveness to ouabain are likely to reflect differences in the density of ouabain receptors rather than differences in binding affinity.

The close relationship between the properties of renal membranes and kidney function provides an unique opportunity to examine the impact of membrane biology on aging processes. Renal function itself is altered by age. Perhaps more importantly, older patients have a decreased capacity to compensate for life-threatening disturbances in fluid and solute homeostasis. In continuing investigations to define the mechanism of renal transport and homeostasis, the plasma membrane of the proximal tubule epithelial cell was found to be differentiated ultrastructurally, biochemically, and functionally into a luminal brush border membrane and an antiluminal basal-lateral membrane. Last year, we reported the development of techniques to isolate the basal-lateral membrane. This preparation, together with an earlier procedure for isolating the brush border membrane, now enable us to examine in model membrane systems the mechanisms by which solutes vectorially enter and leave the tubular cell. We found that the uptakes of L-proline and D-glucose by brush border and basal-lateral membranes represented mostly transport into an intravesicular space rather than membrane binding. A  $\text{Na}^+$  electrochemical gradient (medium-vesicle) stimulated the initial rates of uptake of both the imino acid and sugar, effecting their transient movements into the membrane vesicle against concentration gradients. The uptakes of L-proline and D-glucose by basal-lateral membranes were less responsive to  $\text{Na}^+$  and the relatively small stimulation could largely be accounted for by contamination of the basal-lateral membrane preparation with brush border membranes. These findings are consistent with the hypothesis that the transcellular "active" transports of L-proline and D-glucose consist of a  $\text{Na}^+$  electrochemical gradient-dependent uphill influx at the luminal brush border end and a  $\text{Na}^+$ -independent downhill efflux at the basal-lateral region of the tubule epithelial cell.

Our previous findings that experimentally imposed  $\text{Na}^+$  gradients, independent of a  $\text{Na}^+$  pump, stimulated electrogenically the uptake of D-glucose in renal and intestinal brush border membrane vesicles are consistent with the  $\text{Na}^+$  gradient hypothesis, which postulates that the non-electrolyte is symported with  $\text{Na}^+$  and the  $\text{Na}^+$  electrochemical gradient across the luminal membrane in intact systems provides the energy for the uphill transport of the sugar. In experiments, by others, with bacterial preparations, it was found that a  $\text{H}^+$  gradient could drive the uptake of metabolites. This is in accordance with the chemiosmotic theory of transport, in which the proton motive force, consisting of potential gradient and a pH gradient, is indirectly coupled to and energetically supports translocation. By application of the chemiosmotic formulation to the  $\text{Na}^+$ -dependent transport of D-glucose in renal membranes, the  $\text{Na}^+$  electrochemical potential gradient,  $\Delta\mu_{\text{Na}^+}$ , may be described as

$$\Delta\mu_{\text{Na}^+} = \Delta\psi + \Delta\mu_{[\text{Na}^+]}$$

where  $\Delta\psi$  is the electrical component, proportional to the membrane potential, and  $\Delta\mu_{[\text{Na}^+]}$  is the chemical component, a function of the transmembrane activity ratio of  $\text{Na}^+$ . We have now shown that: the initial rate of D-glucose uptake in brush border membrane vesicles was correlated with the  $\text{Na}^+$  electrochemical potential; each component, the electrical or chemical gradient, when assayed independently, supported the uphill transport of the sugar; and when the two components were combined the rates of D-glucose uptake summated. These findings are consistent with the role of ionic gradients in energizing uphill solute transport and, thus, provide evidence that extends the chemiosmotic theory to the  $\text{Na}^+$  electrochemical gradient-mediated transport of D-glucose in the mammalian kidney.

Specific fluorescent probes, such as 3,3-dipropylthiocarbocyanine iodide, which are lipophilic and ionically charged and thus bind to membranes, but whose binding and intensity of fluorescence are a function of the membrane charge, can effectively monitor membrane potential. Experiments in progress showed a decrease in fluorescence intensity concomitant with the co-transport of  $\text{Na}^+$  and glucose, suggesting that the brush border membrane became depolarized as the solutes were translocated from the outside to the inside of the membrane vesicle.

Last year we reported studies on the mechanisms of amino acid transport systems in renal brush border membrane vesicles showing specific uptake systems for dibasic amino acids, acidic amino acids, neutral amino acids, imino acids (proline + hydroxyproline) and glycine. These findings provide strong experimental evidence for the concept of multiple amino acid transport systems. Further support for this hypothesis was obtained with the finding that the mechanism for transport of  $\beta$ -alanine (and other  $\beta$ -amines, *e.g.* taurine) was different from that for  $\alpha$ -alanine. These observations showed that the luminal segment of the proximal tubule plasma membrane is the site of recognition of  $\beta$ - and  $\alpha$ -amino acids and it may be the locus of the defect in patients with hyper- $\beta$ -alanuria. The two systems for neutral amino acids were similar, however, in that they both were dependent on a  $\text{Na}^+$  electrochemical gradient and were electrogenic processes.

Specific age-related changes in membrane transport systems which are fundamental to renal function were found. In this study, specific activities of enzyme

markers in isolated brush border and basal-lateral membranes and  $\text{Na}^+$ -gradient-dependent and -independent transports of glucose and amino acids in brush border membrane vesicles from mature (1 1/2-7 yr) and aged (11-13 yr) dogs were compared. Trehalase and maltase decreased about 80 and 60%, respectively, in brush border from old dogs.  $\gamma$ -Glutamyltranspeptidase and  $\text{HCO}_3^-$ -stimulated ATPase were not altered, suggesting that the lowered activities of the disaccharidases were highly specific.  $\text{NaK}$ -ATPase in brush border membranes decreased 35% with age, but the activity of the enzyme did not change in basal-lateral membranes. Correlated with the decrement in disaccharidase activities were decreases of 40% in the initial rates of glucose uptake, with and without a  $\text{Na}^+$  gradient. No age-related decrements were found in the initial rates of amino acid transport systems, represented by the uptakes of alanine, glutamate, arginine, and proline, with and without a  $\text{Na}^+$  gradient, hence consistent with an unaltered  $\gamma$ -glutamyltranspeptidase.

Hormones, putative neurotransmitters, and drugs have membrane target sites and their actions, mediated via the cyclic nucleotides, adenosine 3',5'-monophosphate (cyclic AMP) and guanosine 3',5'-monophosphate (cyclic GMP), in regulating ion movements are fundamental to the control of various physiological systems as well as to age-related alterations in these vital functions. Thus, the enzymes that determine the tissue levels of the cyclic nucleotides by synthesis, adenylate cyclase and guanylate cyclase, or by degradation, the phosphodiesterases, and the regulation of these enzyme activities are of great significance.

Cyclic nucleotide phosphodiesterase activity of cerebral cortical extracts was measured with 0.1-100  $\mu\text{M}$  cyclic AMP and cyclic GMP and found to be dependent on both  $\text{Ca}^{2+}$  and added cyclic nucleotides. With decreasing substrate concentration, activity with cyclic GMP became more dependent on  $\text{Ca}^{2+}$  whereas hydrolysis of cyclic AMP became less dependent. Cyclic GMP at 3  $\mu\text{M}$  stimulated the hydrolysis of 0.1-10  $\mu\text{M}$  cyclic AMP in the absence of  $\text{Ca}^{2+}$  ( $<10^{-10}$  M) but inhibited activity with 200  $\mu\text{M}$   $\text{Ca}^{2+}$  present. This differential, substrate- and  $\text{Ca}^{2+}$ -dependent regulation was attributed to the presence of at least two types of phosphodiesterase distinguishable by DEAE-column chromatography. In the absence of  $\text{Ca}^{2+}$ , activity with 1  $\mu\text{M}$  cyclic GMP eluted in one minor peak followed by two major peaks, D-I and D-II. Activity with 1  $\mu\text{M}$  cyclic AMP eluted almost entirely in D-II. Hydrolysis of cyclic AMP in D-II was activated by cyclic GMP. With added  $\text{Ca}^{2+}$  plus a  $\text{Ca}^{2+}$ -dependent regulator (CDR), activity with 1  $\mu\text{M}$  cyclic GMP was markedly increased and eluted entirely at D-I. Total activity with 1  $\mu\text{M}$  cyclic AMP was only moderately increased and eluted as D-I with a shoulder at D-II. Elution profiles with 100  $\mu\text{M}$  substrate were relatively independent of substrate, with D-I predominant with  $\text{Ca}^{2+}$ -CDR present and D-II predominant in its absence. Kinetic analysis of rechromatographed D-I showed a 20- to 40-fold activation by  $\text{Ca}^{2+}$ -CDR that was largely due to an increase in  $V_{\text{max}}$  with only 50% decreases in  $K_m$ . Both substrates competitively inhibited hydrolysis of the other with  $K_i$  values equal to their respective  $K_m$  values (1.7  $\mu\text{M}$  for cyclic GMP and 48  $\mu\text{M}$  for cyclic AMP with  $\text{Ca}^{2+}$ -CDR present). Studies with theophylline and trifluoperazine indicate differential, substrate-dependent inhibitions of both enzymes. These findings demonstrate that phosphodiesterase activity in neural tissue is subject to regulation by  $\text{Ca}^{2+}$ , cyclic GMP, and inhibitors in a complex, substrate-specific and concentration-dependent manner.

Cyclic nucleotide phosphodiesterase in the basal-lateral segment of plasma membranes from proximal tubule cells of the rabbit renal cortex was studied and compared to that in the brush border segment of the plasma membrane. Both cyclic AMP and cyclic GMP were hydrolyzed by the basal-lateral membrane, but activity varied differently with the two substrates in a complex concentration-dependent manner. Activity with cyclic AMP was greater than, equal to, or less than with cyclic GMP, at concentrations of 1000, 100, and 10 to 1  $\mu\text{M}$ , respectively. Basal-lateral membrane phosphodiesterase activities at 1 and 500  $\mu\text{M}$  substrate exhibited differential responses to pH, metals, heat, and a heat stable inhibitor. Stimulation by cyclic GMP and cyclic IMP of cyclic AMP hydrolysis was found in basal-lateral but not in brush border membranes. This stimulation was potentiated by EGTA and EDTA, inhibited by Triton X-100, and totally blocked by  $\text{Zn}^{2+}$ . The findings indicate that multiple forms of phosphodiesterase are present in the basal-lateral segment and these differ from the activities in the brush border region of the plasma membrane. The characteristics of (1) allosteric, cyclic GMP-sensitivity of cyclic AMP phosphodiesterase, and (2) relatively high cyclic GMP phosphodiesterase activity in basal-lateral membranes, which are also enriched in adenylate and guanylate cyclase, suggest an important physiological role for these phosphodiesterases in the regulation of net production of cyclic nucleotides in the renal cortex.

Recent studies suggest that the level of cyclic GMP in tissues is increased by the action of cholinergic and  $\alpha$ -adrenergic agonists, in contradistinction to the action of  $\beta$ -adrenergic agonists which increase the level of cyclic AMP. Synthesis of cyclic GMP is catalyzed by the enzyme guanylate cyclase and this year we focused on this enzyme and the regulation of its activity. Cytosolic guanylate cyclase activity in cell-free preparations of the rabbit renal cortex was increased 3- to 5-fold by catecholamines. The plasma membrane-bound enzyme was not activated, although hormone receptors were present. Stimulation was augmented by  $\text{NaN}_3$ , which by itself had little effect on the soluble enzyme activity. With a partially purified enzyme, activity was enhanced by 0.01 to 0.1  $\mu\text{M}$  1-epinephrine and activated half-maximally by about 1  $\mu\text{M}$ . In decreasing potency, epinephrine > isoproterenol > norepinephrine > dopamine > catechol. Phenylephrine and metanephrine did not stimulate. 1-Epinephrine-stimulation of the enzyme was reversed by dialysis and the deactivated enzyme was reactivatable by a second exposure to the catecholamine. Activation by catecholamines was not stereospecific. Epinephrine-stimulated guanylate cyclase activity in the crude cytosolic fraction was partially inhibited by  $\alpha$ -adrenergic antagonists, but neither  $\alpha$ - nor  $\beta$ -blockers inhibited when the partially purified enzyme was used; thus, leaving open the question of a role for typical  $\alpha$ - or  $\beta$ -adrenergic mechanisms in this regulation of the soluble enzyme. Adrenochrome was the most potent activator of the partially purified guanylate cyclase, being approximately 10-times more effective than epinephrine. Epinephrine and adrenochrome activated in the presence of reducing agents, i.e., ascorbate, DTT and  $\text{N}_2$ , although the enzyme in a more SH-reduced form and in the absence of oxygen had a decreased sensitivity to both effectors. Epinephrine activated soluble guanylate cyclase in several tissues, including cerebrum, cerebellum, brain stem, lung, heart, liver, ductus deferens and colon. The finding that the enzyme was activated by very low concentrations of catecholamines suggests a specific interaction of these agonists with the cytosolic guanylate cyclase system which results in modulation of enzyme activity.



Contraction and relaxation of smooth muscle, induced by hormones, neurotransmitters and drugs, are associated with dramatic changes in the level of cyclic GMP. In view of the crucial importance of the vascular, gastrointestinal, and urogenital systems to the well-being of the aged, the regulation of smooth muscle guanylate cyclase is receiving special attention. We found that guanylate cyclase of the aorta, colon, and ductus deferens had characteristics distinct from the enzyme of non-smooth muscle tissues (liver, kidney, lung, heart). A divalent metal ion was required for enzyme activity, preferably  $Mn^{2+}$ . In smooth muscle,  $Mg^{2+}$  substituted for  $Mn^{2+}$ , whereas in the other tissues  $Mg^{2+}$  was a poor replacement.  $Ca^{++}$ , by itself, could not satisfy the metal ion requirement. However, in smooth muscle,  $Ca^{2+}$  increased the activity of the enzyme in the presence of excess  $Mn^{2+}$ , but inhibited the activity in the presence of excess  $Mg^{2+}$ . The combination of  $Mg^{2+}$  and  $Ca^{2+}$  did not affect activity of the enzyme from non-smooth muscle tissues. Vitamin C and arachidonic acid (a precursor of prostaglandins) activated the enzyme. This activation was blocked by  $Ca^{2+}$ , only when  $Mg^{2+}$  was used as the metal cofactor. Since it is presumed that *in situ*  $Mg^{2+}$  rather than  $Mn^{2+}$  is the "physiological" divalent cation, these findings suggest that in smooth muscle, changes in the flux of  $Ca^{2+}$  serve to modulate the activity of the enzyme and this is somehow coordinated with  $Ca^{2+}$  regulation of excitation-contraction coupling.

Hormones and putative neurotransmitters that induce smooth muscle contraction also enhance the turnover of phosphatidyl inositol in membranes. However, the precise relationship between this action,  $Ca^{2+}$  flux, and cyclic GMP level remains to be determined. Using segments of the ductus deferens, we found that acetylcholine had two effects on phosphatidyl inositol; it decreased the concentration of the phospholipid in the tissue and it increased the rate of phosphatidyl inositol turnover. These actions required the presence of  $Ca^{2+}$  and were blocked by the muscarinic receptor antagonist, atropine, but not by the nicotinic receptor antagonist, tubocurarine. Deletion of  $Ca^{2+}$  from the medium or the depletion of intracellular  $Ca^{2+}$  by the addition of specific  $Ca^{2+}$  ionophores blocked the response.  $Ca^{2+}$  was also required for acetylcholine to provoke increases in cyclic GMP levels. Vasodilators, including hydralazine, nitroprusside,  $NaNO_2$ , and chlorpromazine, also enhanced phosphatidyl inositol turnover, but in contrast to the action of vasoconstrictors, the concentration of the phospholipid was not decreased. In cell-free systems from the ductus deferens we found that the vasodilators activated the synthesis of phosphatidyl inositol. On the other hand,  $Ca^{2+}$  inhibited the enzyme. These preliminary findings suggest that vasoconstrictors, such as cholinergic and  $\alpha$ -adrenergic agonists, activate phosphatidyl inositol breakdown whereas vasodilators enhance the synthesis of the phospholipid and that such actions may be mediated by  $Ca^{2+}$  serving as a "second messenger."

Permeability properties of membranes are thought to be regulated by phosphorylation/dephosphorylation of specific membrane proteins in reactions catalyzed by cyclic AMP-dependent and -independent protein kinases and phosphatases. Last year we reported the presence of at least three protein kinases in the brush border segment of the renal tubule cell plasma membrane. The cyclic AMP-dependent kinase was extracted from the membrane, and its catalytic subunit purified. This preparation now permits us to attempt to identify a specific membrane protein which is phosphorylated as a result of the role of cyclic AMP in mediating the actions of hormones, e.g. parathyroid, calcitonin, catecholamines. In addition, because of findings that in heart muscle cytosolic



can be used as models for the interactions in chromatin, so that the essential features of these interactions can be understood. Polylysine and poly(Lys<sub>70</sub>Ala<sub>30</sub>) lead to one kind of compaction of DNA characterized by highly negative circular dichroism (CD) which we have called the  $\psi(-)$  structure, whereas poly(Lys<sub>50</sub>Ala<sub>50</sub>) and poly(Lys<sub>30</sub>Ala<sub>70</sub>) lead to a different type of compaction characterized by a highly positive CD, which we have called the  $\psi(+)$  structure. Phosphate binding metal ions (e.g. Mg<sup>2+</sup>) enhance the CD effect not only of the  $\psi(-)$  structures, as previously shown, but also of the  $\psi(+)$  structures. Some base binding metal ions and complexes, e.g. Cu<sup>2+</sup> and *cis*[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>] can reversibly convert  $\psi(-)$  structures to  $\psi(+)$ . Other base binding metal ions (e.g. Ag<sup>+</sup>) have the reverse effect;  $\psi(+)$  is converted to  $\psi(-)$ . The varied influences of the metals is illustrated by the fact that *trans*[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>] converts  $\psi(-)$  to  $\psi(+)$  and enhances the CD of  $\psi(+)$  structures; Ag<sup>+</sup> converts  $\psi(+)$  to  $\psi(-)$  and enhances the CD of  $\psi(-)$  structures; *trans*[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>] has an intermediate effect; it diminishes the  $\psi(-)$  CD without conversion to  $\psi(+)$ . The effects of metal ions are generally reversible. Reactions of DNA-polypeptide systems with metal ions are therefore versatile tools for controlling at will the way in which DNA molecules are compacted. The metals reorganize an already organized structure. Neither  $\psi(+)$  nor  $\psi(-)$  structures can be produced by the addition of a Pt complex or Ag<sup>+</sup> ion to solutions of DNA and polypeptides that were directly mixed. Only DNA polypeptide complexes produced by gradient dialysis and thus already poised in an ordered structure are susceptible to the metal ion effects. The  $\psi$  structure is not produced by the addition of polypeptides to metal complexes of DNA. We conclude that polypeptides under certain conditions can organize the DNA molecules into a rigid matrix whose exact orientation depends on the nature of the polypeptide, and which can be perturbed by metals.

The genetic information from the DNA in chromatin is transcribed into RNA in the presence of the enzyme RNA polymerase. The enzyme from *E. coli* requires activation by divalent metal ions; i.e. Mg<sup>2+</sup>, Co<sup>2+</sup>, or Mn<sup>2+</sup>. However, Mn<sup>2+</sup> but not Mg<sup>2+</sup> and Co<sup>2+</sup> induce the "incorrect" incorporation of deoxynucleotide along with the desired ribonucleotide. Such misincorporation of deoxynucleotide into RNA through the agency of metal ions could lead to the kind of error in the structure of an information molecule that could produce deleterious changes in an organism. It was postulated that Co<sup>2+</sup> and Mg<sup>2+</sup> bound enzymes have conformations sensitive to the difference between ribo- and deoxy-nucleotides, and that Mn<sup>2+</sup> bound enzyme has a different conformation that lacks such sensitivity. Evidence for this hypothesis was obtained from uv and ORD melting curves. The Co<sup>2+</sup> and Mn<sup>2+</sup> enzymes exhibited denaturing transitions at lower temperatures than the Mg<sup>2+</sup> enzyme. Such differences in transition temperature generally reflect differences in conformation.

Among the potentially most destructive effects of metal ions on nucleic acids is their ability to degrade RNA. Certain metal ions are exceedingly effective in this degradation; among them are Zn<sup>2+</sup>, Pb<sup>2+</sup>, and Ce<sup>3+</sup>. In the case of Zn<sup>2+</sup> the degradation stops at the level of the mononucleotide; the latter is not further dephosphorylated to a nucleoside. Such dephosphorylation does occur with Pb<sup>2+</sup> and Ce<sup>3+</sup>. We therefore asked why Zn<sup>2+</sup> ions can act on internucleotide bonds but not on the single phosphate on a mononucleotide, and why, in contrast, Pb<sup>2+</sup> and Ce<sup>3+</sup> can go on to remove this last phosphate.

Of these three metals  $Ce^{3+}$  is the most active dephosphorylating agent and  $Zn^{2+}$  is least active, with  $Pb^{2+}$  intermediate. The great difference between  $Ce^{3+}$  and  $Pb^{2+}$  in reactivity toward adenine nucleotides correlates very well with the CD spectra of the complexes. Nonreactive zinc complexes have very intense CD spectra, while highly reactive cerium complexes have virtually no CD spectrum. The intense CD spectra of the  $Zn^{2+}$  complexes indicate a high degree of nucleotide stacking, and we conclude that this stacking protects the phosphate groups and prevents dephosphorylation. In contrast, the lack of stacking of  $Ce^{3+}$  complexes provides no inhibition for the reaction.  $Pb^{2+}$  produces extensive dephosphorylation, although less than  $Ce^{3+}$ ; nevertheless the  $Pb^{2+}$  complexes have significant CD spectra. To understand the differences between the  $Pb^{2+}$  and  $Zn^{2+}$  complexes requires a more thorough analysis in which pH is taken into consideration. The pH profile for  $Pb(II)$  3'AMP, for example, shows that the CD effect is maximal at low pH and almost obliterated at pH 7. Above pH 8 extensive hydrolysis of Pb occurs. There is thus a window around pH 7 in which neither extensive stacking nor metal hydrolysis limit the reactivity of the phosphate group, permitting dephosphorylation. pH 7 is in fact the optimal pH for the reaction, which is also favored at this pH by solubility considerations. Similar analyses have been made of other  $Pb(II)$  nucleotide complexes and account for their reactivity. Such analyses of Zn complexes show that there is no window for the dephosphorylation reaction. The ability of metal ions to promote dephosphorylation depends on the interplay of many factors, including the presence of a hydroxyl group adjacent to the phosphate to be cleaved, lack of nucleotide stacking, metal hydrolysis, and solubility of the complex. The ability of metal ions to dephosphorylate nucleotides obviously can be a factor in their toxicity.

The major use of man made or man modified macromolecules are based on their mechanical properties and their inertness. Nevertheless, modifications to obtain reactivity lead to very useful compounds. Reactive water soluble macromolecules have been synthesized and their effects on mammalian cells in culture studied, the main emphasis being on the interaction of the macromolecules with cell membrane. Dextran, a natural polysaccharide, is biologically inert - it is neither bound nor consumed by cells; to this compound were attached highly reactive groups and the effects of these derivatives on mouse cells growing in culture were studied. The following were used as reactive groups: (a) mercury (II) compounds, which bind very strongly to sulfhydryl groups; (b) diazonium compounds, which react with tyrosine and histidine residues of proteins; (c) organic dyes, which bind nucleic acids; (d) the organic dye, Rose Bengal, which in the presence of light turns oxygen from the normal, inert triplet state into the very short-lived and extremely toxic singlet state. The substituted dextrans (a)-(c) represent reagents which bind strongly and selectively to some components of the surface of the living cell; the last compound (d) in the presence of light transforms oxygen so that it destroys the binding site of the compound.

Studies on the oxygenation, oxidation and metal ion affinities of certain abnormal hemoglobins with single amino acid substitutions demonstrate that the  $Zn(II)$  binding site which increases the oxygen affinity of hemoglobin and the  $Cu(II)$  binding site associated with the rapid oxidation of hemoglobin both involve histidine  $\beta$ -143. A decrease in the effect of zinc is coupled

to an increase in the level of 2,3-diphosphoglyceric acid (DPG), a substance thought to play a major role in regulating the physiological oxygen affinity of hemoglobin. This finding is explained by an overlap in the binding site for zinc and DPG. Electron spin resonance studies with Cu(II) show that the Cu(II) is actually bound prior to the oxidation. The location of the Cu(II) binding site some distance from the heme suggests an electron transfer process through a region of the protein moiety.

The erythrocytes of older individuals are found to be slightly more fragile, with a wider and more asymmetric distribution of fragilities. However, although the cells of older individuals are more fragile, their rate of hemolysis is slower. While these changes do not seem to indicate any dramatic instability of erythrocytes in older individuals, they do indicate significant changes in the structure of the cell membrane and/or the intracellular composition of the cells. In an attempt to understand some of the possible factors which can affect the fragility distribution, we have found that there is no simple correlation between the distribution of fragilities and the distribution of cell ages.

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CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS					
SUMMARY OF WORK (200 words or less - underline keywords)					
<p>This study of <u>membrane transport</u> is targeted to provide the basic scientific information needed to: determine the mechanisms by which <u>physiological control systems</u> are altered in the aged; and use this new information as a base for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities. <u>Membrane vesicles</u> derived from the luminal <u>brush border</u> segment and the antiluminal <u>basal-lateral</u> region of the <u>renal tubule epithelial cell plasma membrane</u> are used as model systems. Topics investigated include: 1) the role of the <u>membrane potential</u> in the <u>Na<sup>+</sup> gradient-dependent uptake of D-glucose</u> by brush border membrane vesicles; 2) the mechanisms and specificities of <u>amino acid transport</u> systems; 3) the comparison of the mechanism by which solutes enter and exit the tubular epithelial cell; 4) the isolation and characterization of the <u>Na<sup>+</sup> gradient-dependent glucose carrier</u> by <u>reconstitution of synthetic membranes</u>; 5) the <u>comparison of enzyme activities and transport properties</u> of membrane vesicles derived from <u>kidneys of mature and aged dogs</u>.</p>					

## Project Description:

**Objectives:** These studies are targeted to define the biochemical and biophysical mechanisms whereby age-dependent changes in membrane transport perturb physiological control systems which lead to a failure to maintain homeostasis. The investigations impact on vital functions, including those in the kidney, brain, heart, gastrointestinal and urogenital tracts. For example, a typical experimental question may be: Why does the kidney of the aged have a decreased capacity to compensate for life-threatening disturbances in fluid and solute homeostasis? The thrust of the work is focused on questions dealing with the biology of membranes, including: (1) molecular organization; (2) role in selective vectorial transport; (3) hormonal regulation of function; (4) catalytic function; (5) turnover; and (6) failure to maintain structure, leading to cell death.

**Methods Employed:** Membrane vesicles derived from the luminal brush border segment and the antiluminal basal-lateral region of the renal tubule epithelial cell plasma membrane are used as model systems. Components of the membrane are resolved and reconstituted to determine whether defects are due to changes in a specific constituent or in the membrane milieu.

**Major Findings:** Last year, we reported the development of techniques to isolate the basal-lateral membrane. This preparation, together with an earlier procedure for isolating the brush border membrane, now enable us to examine in model membrane systems the mechanisms by which solutes vectorially enter and leave the tubular cell. We found that the uptakes of L-proline and D-glucose by brush border and basal-lateral membranes represented mostly transport into an intravesicular space rather than membrane binding. A  $\text{Na}^+$  electrochemical gradient (medium > vesicle) stimulated the initial rates of uptake of both the imino acid and sugar, effecting their transient movements into the membrane vesicle against concentration gradients. The uptakes of L-proline and D-glucose by basal-lateral membranes were less responsive to  $\text{Na}^+$  and the relatively small stimulation could largely be accounted for by contamination of the basal-lateral membrane preparation with brush border membranes. These findings are consistent with the hypothesis that the transcellular "active" transports of L-proline and D-glucose consist of a  $\text{Na}^+$  electrochemical gradient-dependent uphill influx at the luminal brush border end and a  $\text{Na}^+$ -independent downhill efflux at the basal-lateral region of the tubule epithelial cell.

Our previous findings that experimentally imposed  $\text{Na}^+$  gradients, independent of a  $\text{Na}^+$  pump, stimulated electrogenically the uptake of D-glucose in renal and intestinal brush border membrane vesicles are consistent with the  $\text{Na}^+$  gradient hypothesis, which postulates that the non-electrolyte is symported with  $\text{Na}^+$  and the  $\text{Na}^+$  electrochemical gradient across the luminal membrane in intact systems provides the energy for the uphill transport of the sugar. In experiments, by others, with bacterial preparations, it was found that a  $\text{H}^+$  gradient could drive the uptake of metabolites. This is in accordance with the chemiosmotic theory of transport, in which the proton motive force, consisting of potential gradient and a pH gradient, is indirectly coupled to

and energetically supports translocation. By application of the chemiosmotic formulation to the  $\text{Na}^+$ -dependent transport of D-glucose in renal membranes, the  $\text{Na}^+$  electrochemical potential gradient,  $\Delta\mu_{\text{Na}^+}$ , may be described as

$$\Delta\mu_{\text{Na}^+} = \Delta\psi + \Delta\mu_{[\text{Na}^+]}$$

where  $\Delta\psi$  is the electrical component, proportional to the membrane potential, and  $\Delta\mu_{[\text{Na}^+]}$  is the chemical component, a function of the transmembrane activity ratio of  $\text{Na}^+$ . We have now shown that: the initial rate of D-glucose uptake in brush border membrane vesicles was correlated with the  $\text{Na}^+$  electrochemical potential; each component, the electrical or chemical gradient, when assayed independently, supported the uphill transport of the sugar; and when the two components were combined the rates of D-glucose uptake summated. These findings are consistent with the role of ionic gradients in energizing uphill solute transport and, thus, provide evidence that extends the chemiosmotic theory to the  $\text{Na}^+$  electrochemical gradient-mediated transport of D-glucose in the mammalian kidney.

Specific fluorescent probes, such as 3,3-dipropylthiocarbocyanine iodide, which are lipophilic and ionically charged and thus bind to membranes, but whose binding and intensity of fluorescence are a function of the membrane charge, can effectively monitor membrane potential. Experiments in progress showed a decrease in fluorescence intensity concomitant with the co-transport of  $\text{Na}^+$  and glucose, suggesting that the brush border membrane became depolarized as the solutes were translocated from the outside to the inside of the membrane vesicle.

Last year we reported studies on the mechanisms of amino acid transport systems in renal brush border membrane vesicles showing specific uptake systems for dibasic amino acids, acidic amino acids, neutral amino acids, imino acids (proline + hydroxyproline) and glycine. These findings provide strong experimental evidence for the concept of multiple amino acid transport systems. Further support for this hypothesis was obtained with the finding that the mechanism for transport of  $\beta$ -alanine (and other  $\beta$ -amines, *e.g.* taurine) was different from that for  $\alpha$ -alanine. These observations showed that the luminal segment of the proximal tubule plasma membrane is the site of recognition of  $\beta$ - and  $\alpha$ -amino acids and it may be the locus of the defect in patients with hyper- $\beta$ -alanuria. The two systems for neutral amino acids were similar, however, in that they both were dependent on a  $\text{Na}^+$  electrochemical gradient and were electrogenic processes.

Studies were initiated to isolate the  $\text{Na}^+$  gradient-dependent glucose carrier and then reconstitute the protein into a functional synthetic phospholipid membrane vesicle. This problem is proving exceedingly difficult because the only available assay is transport function itself rather than catalytic activity, as in the successful reconstitution studies wherein  $\text{Na}^+$  K<sup>+</sup> ATPase and  $\text{Ca}^{2+}$  ATPase activities were monitored prior to reincorporation into vesicles capable of transporting  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$ , respectively. Nevertheless considerable progress was made in establishing conditions, such as: type of detergent to solubilize the membrane, detergent concentration and detergent/protein ratios, time of sonication, method of detergent removal, buffer,



pH, temperature, protecting agents, and phospholipid composition. In addition, many of the techniques traditionally used for purification of soluble proteins were evaluated with protein "solubilized" from membranes.

Specific age-related changes in membrane transport systems which are fundamental to renal function were found. In this study, specific activities of enzyme markers in isolated brush border and basal-lateral membranes and  $\text{Na}^+$ -gradient-dependent and -independent transports of glucose and amino acids in brush border membrane vesicles from mature (1 1/2-7 yr) and aged (11-13 yr) dogs were compared. Trehalase and maltase decreased about 80 and 60%, respectively, in brush borders from old dogs.  $\gamma$ -Glutamyltranspeptidase and  $\text{HCO}_3^-$ -stimulated ATPase were not altered, suggesting that the lowered activities of the disaccharidases were highly specific.  $\text{Na}^+$ -ATPase in brush border membranes decreased 35% with age, but the activity of the enzyme did not change in basal-lateral membranes. Correlated with the decrement in disaccharidase activities were decreases of 40% in the initial rates of glucose uptake, with and without a  $\text{Na}^+$  gradient. No age-related decrements were found in the initial rates of amino acid transport systems, represented by the uptakes of alanine, glutamate, arginine, and proline, with and without a  $\text{Na}^+$  gradient, hence consistent with an unaltered  $\gamma$ -glutamyltranspeptidase.

#### Significance to Biomedical Research and to the Program of the Institute:

These studies, using renal plasma membrane vesicles as model membranes to investigate transport processes, describe mechanisms whereby age-dependent perturbation in physiological control systems may lead to the inability of the aged organism to maintain homeostasis. This fundamental information is needed for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities.

#### Proposed Course of the Project: Studies on the mechanisms of transport will:

(1) further probe the interrelations between membrane potential and active solute transport, (2) examine  $\text{Na}^+$ - $\text{H}^+$  exchange systems as a mechanism of acid-base and salt-water balance, (3) define in membrane vesicle systems the mechanisms of anion transport, including those for chloride, phosphate and bicarbonate, (4) determine how these transport systems are regulated, (5) explore the recent technique of membrane resolution-reconstitution to define transport systems in terms of molecular organization of the membrane, and (6) continue to apply these fundamental studies to transport systems in the kidney of the aged dog.

#### Publications:

Fass, S. J., Hammerman, M. R. and Sacktor, B.: Transport of amino acids in renal brush border membrane vesicles. Uptake of the neutral amino acid L-alanine. J. Biol. Chem. 252: 583-590, 1977.

Hammerman, M. R. and Sacktor, B.: Transport of amino acids in renal brush border membrane vesicles. Uptake of L-proline. J. Biol. Chem. 252: 591-595, 1977.

Liang, C. T. and Sacktor, B.: Bicarbonate-stimulated ATPase in the renal proximal tubule luminal (brush border) membrane. Arch. Biochem. Biophys. 176: 285-297, 1976.

Liang, T. and Sacktor, B.: Preparation of renal cortex basal-lateral and brush border membranes. Localization of adenylate cyclase and guanylate cyclase activity. Biochim. Biophys. Acta 466: 474-487, 1977.

Sacktor, B.: The Brush Border of the Proximal Renal Tubule and the Intestinal Mucosa. In Jamieson, G. A. and Robinson, D. M. (Eds.): Mammalian Cell Membranes, London, Butterworths, 1977, Vol. 4, pp. 221-254.

Sacktor, B.: Transport in Membrane Vesicles Isolated from the Mammalian Kidney and Intestine. In Sanadi, R. (Ed.): Current Topics in Bioenergetics, New York, Academic Press, 1977, Vol. 6, pp. 39-81.

Sacktor, B.: Mechanisms and specificities of amino acid transport in proximal tubule luminal membrane vesicles. Proceedings of the Macy Conference on Renal Function, in press.

Slack, E. N., Liang, C-C. T. and Sacktor, B.: Transport of L-proline and D-glucose in luminal (brush border) and contraluminal (basal-lateral) membrane vesicles from the renal cortex. Biochem. Biophys. Res. Commun., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00042-04 LMA
PERIOD COVERED <b>July 1, 1976 to September 30, 1977</b>		
TITLE OF PROJECT (80 characters or less) <b>Physiological Control Systems and Aging II          Mechanisms of Hormonal Regulation</b>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:  OTHER:	B. Sacktor C. Filburn T. Liang J. Ketley R. Balakir T. Takenawa K. Egawa	Chief, Lab. Molec. Aging Staff Fellow Staff Fellow Staff Fellow Chemist Visiting Fellow (EOD 9/12/76) Visiting Assoc. (EOD 3/27/77)
		LMA NIA LMA NIA LMA NIA LMA NIA LMA NIA LMA NIA LMA NIA
COOPERATING UNITS (if any) H. Metzger, Hoechst Aktiengesellschaft, Pharma Biochemie, Frankfurt, West Germany G. Wyatt, Dept. Biology, Yale University, New Haven, CT.		
LAB/BRANCH  SC	Gerontology Research Center, Laboratory of Molecular Aging  Intermediary Metabolism Section	
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS: 7.5	PROFESSIONAL: 5.5	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <p>           This study of <u>hormonal regulation</u> is targeted to define the <u>biochemical mechanisms</u>            whereby <u>age-dependent</u> changes perturb <u>physiological control systems</u> and, thus,            lead to a failure to maintain homeostasis in the aged. Investigations are            focused on the biochemical interactions of hormones, which are mediated via  <u>cyclic AMP</u> and <u>cyclic GMP</u>. Topics investigated include : 1) <u>hormone receptors</u>            in membranes; 2) <u>adenylate cyclase</u> and <u>guanylate cyclase activities</u>; 3) phos-            phorylation of membrane proteins by cyclic nucleotide-dependent and -independent  <u>protein kinases</u>; 4) control of cyclic nucleotide levels by regulation of  <u>phosphodiesterase</u> activities. Hormonal regulatory systems in <u>kidney</u>, <u>heart</u>,  <u>brain</u>, <u>colon</u>, <u>aorta</u>, and <u>ductus deferens</u> were studied.         </p>		

## Project Description:

**Objectives:** These studies are targeted to define the biochemical mechanisms whereby age-dependent changes in hormonal regulation perturb physiological control systems and, thus, lead to a failure to maintain homeostasis in the aged. This new information is needed as a base for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities. Investigations are focused on the biochemical interactions of hormones, mediated via cyclic AMP and cyclic GMP. The research has impact on the mechanism underlying age-related changes in renal, neural, cardiac and smooth muscle function.

**Methods Employed:** Membrane and cytosol preparations derived from kidney, brain, heart, aorta, colon and ductus deferens were used.

**Major Findings:** Hormones, putative neurotransmitters, and drugs have membrane target sites and their actions, mediated via the cyclic nucleotides, adenosine 3',5'-monophosphate (cyclic AMP) and guanosine 3',5'-monophosphate (cyclic GMP), in regulating ion movements are fundamental to the control of various physiological systems as well as to age-related alterations in these vital functions. Thus, the enzymes that determine the tissue levels of the cyclic nucleotides by synthesis, adenylate cyclase and guanylate cyclase, or by degradation, the phosphodiesterases, and the regulation of these enzyme activities are of great significance.

Cyclic nucleotide phosphodiesterase activity of cerebral cortical extracts was measured with 0.1-100  $\mu\text{M}$  cyclic AMP and cyclic GMP and found to be dependent on both  $\text{Ca}^{2+}$  and added cyclic nucleotides. With decreasing substrate concentration, activity with cyclic GMP became more dependent on  $\text{Ca}^{2+}$  whereas hydrolysis of cyclic AMP became less dependent. Cyclic GMP at 3  $\mu\text{M}$  stimulated the hydrolysis of 0.1-10  $\mu\text{M}$  cyclic AMP in the absence of  $\text{Ca}^{2+}$  ( $<10^{-10}$  M) but inhibited activity with 200  $\mu\text{M}$   $\text{Ca}^{2+}$  present. This differential, substrate- and  $\text{Ca}^{2+}$ -dependent regulation was attributed to the presence of at least two types of phosphodiesterase distinguishable by DEAE-column chromatography. In the absence of  $\text{Ca}^{2+}$ , activity with 1  $\mu\text{M}$  cyclic GMP eluted in one minor peak followed by two major peaks, D-I and D-II. Activity with 1  $\mu\text{M}$  cyclic AMP eluted almost entirely in D-II. Hydrolysis of cyclic AMP in D-II was activated by cyclic GMP. With added  $\text{Ca}^{2+}$  plus a  $\text{Ca}^{2+}$ -dependent regulator (CDR), activity with 1  $\mu\text{M}$  cyclic GMP was markedly increased and eluted entirely at D-I. Total activity with 1  $\mu\text{M}$  cyclic AMP was only moderately increased and eluted as D-I with a shoulder at D-II. Elution profiles with 100  $\mu\text{M}$  substrate were relatively independent of substrate, with D-I predominant with  $\text{Ca}^{2+}$ ·CDR present and D-II predominant in its absence. Kinetic analysis of rechromatographed D-I showed a 20- to 40-fold activation by  $\text{Ca}^{2+}$ ·CDR that was largely due to an increase in  $V_{\text{max}}$  with only 50% decreases in  $K_m$ . Both substrates competitively inhibited hydrolysis of the other with  $K_i$  values equal to their respective  $K_m$  values (1.7  $\mu\text{M}$  for cyclic GMP and 48  $\mu\text{M}$  for cyclic AMP with  $\text{Ca}^{2+}$ ·CDR present). Studies with theophylline and trifluoperazine indicate differential, substrate-dependent inhibitions of both enzymes. These findings demonstrate that phosphodiesterase activity in neural tissue is subject to

regulation by  $\text{Ca}^{2+}$ , cyclic GMP, and inhibitors in a complex, substrate-specific and concentration-dependent manner.

Cyclic nucleotide phosphodiesterase in the basal-lateral segment of plasma membranes from proximal tubule cells of the rabbit renal cortex was studied and compared to that in the brush border segment of the plasma membrane. Both cyclic AMP and cyclic GMP were hydrolyzed by the basal-lateral membrane, but activity varied differently with the two substrates in a complex concentration-dependent manner. Activity with cyclic AMP was greater than, equal to, or less than with cyclic GMP, at concentrations of 1000, 100, and 10 to 1  $\mu\text{M}$ , respectively. Basal-lateral membrane phosphodiesterase activities at 1 and 500  $\mu\text{M}$  substrate exhibited differential responses to pH, metals, heat, and a heat stable inhibitor. Stimulation by cyclic GMP and cyclic IMP of cyclic AMP hydrolysis was found in basal-lateral but not in brush border membranes. This stimulation was potentiated by EGTA and EDTA, inhibited by Triton X-100, and totally blocked by  $\text{Zn}^{2+}$ . The findings indicate that multiple forms of phosphodiesterase are present in the basal-lateral segment and these differ from the activities in the brush border region of the plasma membrane. The characteristics of (1) allosteric, cyclic GMP-sensitivity of cyclic AMP phosphodiesterase, and (2) relatively high cyclic GMP phosphodiesterase activity in basal-lateral membranes, which are also enriched in adenylate and guanylate cyclase, suggest an important physiological role for these phosphodiesterases in the regulation of net production of cyclic nucleotides in the renal cortex.

Recent studies suggest that the level of cyclic GMP in tissues is increased by the action of cholinergic and  $\alpha$ -adrenergic agonists, in contradistinction to the action of  $\beta$ -adrenergic agonists which increase the level of cyclic AMP. Synthesis of cyclic GMP is catalyzed by the enzyme guanylate cyclase and this year we focused on this enzyme and the regulation of its activity. Cytosolic guanylate cyclase activity in cell-free preparations of the rabbit renal cortex was increased 3- to 5-fold by catecholamines. The plasma membrane-bound enzyme was not activated, although hormone receptors were present. Stimulation was augmented by  $\text{NaN}_3$ , which by itself had little effect on the soluble enzyme activity. With a partially purified enzyme, activity was enhanced by 0.01 to 0.1  $\mu\text{M}$  1-epinephrine and activated half-maximally by about 1  $\mu\text{M}$ . In decreasing potency, epinephrine > isoproterenol > norepinephrine > dopamine > catechol. Phenylephrine and metanephrine did not stimulate. 1-Epinephrine-stimulation of the enzyme was reversed by dialysis and the deactivated enzyme was reactivatable by a second exposure to the catecholamine. Activation by catecholamines was not stereospecific. Epinephrine-stimulated guanylate cyclase activity in the crude cytosolic fraction was partially inhibited by  $\alpha$ -adrenergic antagonists, but neither  $\alpha$ - nor  $\beta$ -blockers inhibited when the partially purified enzyme was used; thus, leaving open the question of a role for typical  $\alpha$ - or  $\beta$ -adrenergic mechanisms in this regulation of the soluble enzyme. Adrenochrome was the most potent activator of the partially purified guanylate cyclase, being approximately 10-times more effective than epinephrine. Epinephrine and adrenochrome activated in the presence of reducing agents, i.e., ascorbate, DTT and  $\text{N}_2$ , although the enzyme in a more SH-reduced form and in the absence of oxygen

had a decreased sensitivity to both effectors. Epinephrine activated soluble guanylate cyclase in several tissues, including cerebrum, cerebellum, brain stem, lung, heart, liver, ductus deferens and colon. The finding that the enzyme was activated by very low concentrations of catecholamines suggests a specific interaction of these agonists with the cytosolic guanylate cyclase system which results in modulation of enzyme activity.

Procedures were developed for the purification of the soluble guanylate cyclase. A purified enzyme is necessary to determine if the hormone stimulation is an intrinsic property of the enzyme protein and the nature of the hormone-protein interaction. Guanylate cyclase from the colon was chosen both because of its high enzymatic specific activity and for its known epinephrine responsiveness. We succeeded in optimizing the conditions for an ammonium sulfate precipitation step, ion-exchange chromatography of DEAE Bio-Gel A and affinity chromatography on butyl agarose and on a mercuryl column, Affi-Gel 50. Initial results from chromatography on a biospecific affinity column, GMP-agarose looked promising, but further modification is required. The enzyme is included during Sephadex G-200 chromatography allowing a molecular weight estimate of 200,000. Large losses of enzyme activity upon freezing or upon chromatography were observed. It is possible that the colon enzyme is in the "activated" form and is labile. This lability of the colon enzyme make purification to homogeneity difficult.

Contraction and relaxation of smooth muscle, induced by hormones, neurotransmitters and drugs, are associated with dramatic changes in the level of cyclic GMP. In view of the crucial importance of the vascular, gastrointestinal, and urogenital systems to the well-being of the aged, the regulation of smooth muscle guanylate cyclase is receiving special attention. We found that guanylate cyclase of the aorta, colon, and ductus deferens had characteristics distinct from the enzyme of non-smooth muscle tissues (liver, kidney, lung, heart). A divalent metal ion was required for enzyme activity, preferably  $Mn^{2+}$ . In smooth muscle,  $Mg^{2+}$  substituted for  $Mn^{2+}$ , whereas in the other tissues  $Mg^{2+}$  was a poor replacement.  $Ca^{2+}$ , by itself, could not satisfy the metal ion requirement. However, in smooth muscle,  $Ca^{2+}$  increased the activity of the enzyme in the presence of excess  $Mn^{2+}$ , but inhibited the activity in the presence of excess  $Mg^{2+}$ . The combination of  $Mg^{2+}$  and  $Ca^{2+}$  did not affect activity of the enzyme from non-smooth muscle tissues. Vitamin C and arachidonic acid (a precursor of prostaglandins) activated the enzyme. This activation was blocked by  $Ca^{2+}$ , only when  $Mg^{2+}$  was used as the metal cofactor. Since it is presumed that *in situ*  $Mg^{2+}$  rather than  $Mn^{2+}$  is the "physiological" divalent cation, these findings suggest that in smooth muscle, changes in the flux of  $Ca^{2+}$  serve to modulate the activity of the enzyme and this is somehow coordinated with  $Ca^{2+}$  regulation of excitation-contraction coupling.

Hormones and putative neurotransmitters that induce smooth muscle contraction also enhance the turnover of phosphatidyl inositol in membranes. However, the precise relationship between this action,  $Ca^{2+}$  flux, and cyclic GMP level remains to be determined. Using segments of the ductus deferens, we found that acetylcholine had two effects on phosphatidyl inositol; it decreased the concentration of the phospholipid in the tissue and it increased the rate of phosphatidyl inositol turnover. These actions required the presence of  $Ca^{2+}$ .

and were blocked by the muscarinic receptor antagonist, atropine, but not by the nicotinic receptor antagonist, tubocurarine. Deletion of  $\text{Ca}^{2+}$  from the medium or the depletion of intracellular  $\text{Ca}^{2+}$  by the addition of specific  $\text{Ca}^{2+}$  ionophores blocked the response.  $\text{Ca}^{2+}$  was also required for acetylcholine to provoke increases in cyclic GMP levels. Vasodilators, including hydralazine, nitroprusside,  $\text{NaNO}_2$ , and chlorpromazine, also enhanced phosphatidyl inositol turnover, but in contrast to the action of vasoconstrictors, the concentration of the phospholipid was not decreased. In cell-free systems from the ductus deferens we found that the vasodilators activated the synthesis of phosphatidyl inositol. On the other hand,  $\text{Ca}^{2+}$  inhibited the enzyme. These preliminary findings suggest that vasoconstrictors, such as cholinergic and  $\alpha$ -adrenergic agonists, activate phosphatidyl inositol breakdown whereas vasodilators enhance the synthesis of the phospholipid and that such actions may be mediated by  $\text{Ca}^{2+}$  serving as a "second messenger."

Permeability properties of membranes are thought to be regulated by phosphorylation/dephosphorylation of specific membrane proteins in reactions catalyzed by cyclic AMP-dependent and -independent protein kinases and phosphatases. Last year we reported the presence of at least three protein kinases in the brush border segment of the renal tubule cell plasma membrane. The cyclic AMP-dependent kinase was extracted from the membrane, and its catalytic subunit purified. This preparation now permits us to attempt to identify a specific membrane protein which is phosphorylated as a result of the role of cyclic AMP in mediating the actions of hormones, e.g. parathyroid, calcitonin, catecholamines. In addition, because of findings that in heart muscle cytosolic cyclic AMP-dependent protein kinases can phosphorylate a specific protein in the sarcoplasmic reticulum membrane and, thus, enhance  $\text{Ca}^{2+}$  transport, the soluble protein kinase activity of the renal cortex was examined. The cytosol contained 60% of the total kinase activity of the cortex, several kinases were identified, separated, and partially characterized. The dominant activity in the cytosol was cyclic AMP-dependent, in contrast to the situation in the brush border membrane wherein most of the activity was cyclic AMP-independent. Thus, we now have two preparations, one derived from the brush border membrane and the other from the cytosol, to examine further the mechanisms by which hormones regulate membrane transport processes.

Studies on the effect of catecholamine treatment on the state of activation of cAMP-dependent protein kinase in rat heart septa were initiated. During perfusion the septa are freeze clamped, stored at  $-70^\circ \text{C}$ , powdered while frozen, and the samples of the frozen powder homogenized and assayed for kinase activity with histones as substrate in the presence and absence of cAMP and a specific inhibitor of cAMP-dependent protein kinase. Initial studies indicated that this method, along with determinations of cAMP levels in the powdered septa, could serve as a good indication of the response of perfused septa to catecholamine treatment and, thus, provide a means of assessing the nature of reduced catecholamine responsiveness in the aged myocardium.

Studies were initiated comparing mature (1 1/2-7 yr) and aged (11-13 yr) dogs for hormonal (parathyroid, calcitonin, catecholamine, vasopressin, and

prostaglandins) activation of adenylate cyclase from basal-lateral and brush border membranes of the renal cortex. Also compared were cyclic AMP-dependent and -independent protein kinases in the two membrane preparations.

Significance to Biomedical Research and to the Program of the Institute:

These studies define the mechanisms whereby age-dependent perturbation in physiological control systems may lead to the inability of the aged organism to maintain homeostasis. This fundamental information is needed for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities.

Proposed Course of the Project: Studies on mechanisms of hormone regulation will focus on: (1) the mechanism of regulation of guanylate cyclase by catecholamines, (2) the action of vasoconstrictors and vasodilators on smooth muscle contraction-relaxation and the roles of  $\text{Ca}^{2+}$ , membrane phospholipids, and cyclic nucleotides, (3) the mechanisms by which the state of phosphorylation-dephosphorylation of specific membrane proteins, a process distal to hormonal reception, regulates transport processes, (4) the mechanism of the reduced catecholamine responsiveness in the aged myocardium, (5) the mechanism of the decreased responsiveness of the caudate nucleus of substantia nigra-lesioned aged rats, (6) the kinetics of the  $\text{Ca}^{2+}$ -regulatory protein cyclic nucleotide phosphodiesterase and the mechanism of action of phenothiazines, (7) the mechanism by which  $\text{Ca}^{2+}$  transport in the aged heart sarcoplasmic reticulum can be enhanced, and (8) application of these fundamental studies on the hormonal responsiveness in the aged dog.

Publications:

Filburn, C. R. and Wyatt, G. R.: Adenylate and guanylate cyclases of *cecropia* silkworm fat body. J. Insect Physiol. 22: 1635-1640, 1976.

Filburn, C., Colpo, F. and Sacktor, B.: Regulation of cyclic nucleotide phosphodiesterases of cerebral cortex by  $\text{Ca}^{2+}$  and cyclic GMP. J. Neurochem., in press.

Filburn, C. R., Karn, J. and Wyatt, G. R.: Cyclic nucleotide phosphodiesterases of *cecropia* silkworm fat body. Biochim. Biophys. Acta, 481: 152-163, 1977.

Filburn, C. R., Liang, C. T. and Sacktor, B.: Cyclic nucleotide phosphodiesterases of the renal cortex. Characterization of basal-lateral membrane activities. J. Membrane Biol., in press.

George, E. R., Balakir, R. A., Filburn, C. F. and Sacktor, B.: Cyclic adenosine monophosphate-dependent and -independent protein kinase activity of renal brush border membranes. Arch. Biochem. Biophys. 180: 429-443, 1977.

Sacktor, B.: Regulation of cyclic AMP and cyclic GMP metabolism in renal cortex tubule cells. Proceedings of the Macy Conference on Renal Function, in press.



Sacktor, B., Balakir, R. A. and Filburn, C. R.: Cyclic adenosine 3',5'-monophosphate-dependent and -independent protein kinases of renal brush border membranes. Solubilization, separation and characterization of multiple forms. Arch. Biochem. Biophys., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF</b> INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00043-04 LMA
PERIOD COVERED July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (80 characters or less) Physiological Control Systems and Aging III Regulation of Intermediary Metabolism		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:  OTHER:	B. Sacktor R. Hansford B. Bulos	Chief, Lab. Molec. Aging Visiting Scientist Research Chemist   LMA NIA LMA NIA LMA NIA
COOPERATING UNITS (if any)  None		
LAB/BRANCH Gerontology Research Center, Laboratory of Molecular Aging		
S: Intermediary Metabolism Section		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS: 4.4	PROFESSIONAL: 2.2	OTHER: 2.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) These studies are targeted to define the biochemical mechanisms whereby age-dependent changes in <u>regulation of intermediary metabolism</u> perturb physiological control systems and, thus, lead to a failure to maintain homeostasis in the aged. Topics include: 1) effect of age on the <u>regulation of the carbohydrate and fat oxidation</u> ; 2) mechanism of control of <u>pyruvate dehydrogenase activity</u> by phosphorylation/dephosphorylation reactions; 3) <u>loci of the defect in fatty acid oxidation in heart mitochondria</u> from aged animals; and 4) mechanism of regulation of the <u>tricarboxylate cycle</u> , the major energy-yielding pathway.		

## Project Description:

Objectives: These studies are targeted to define the biochemical mechanisms whereby age-dependent changes in regulation of intermediary metabolism perturb physiological control systems and, thus, lead to a failure to maintain homeostasis in the aged. This new information is needed as a base for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities. The thrust of the work is focused on mitochondrial metabolism and the regulation of the interaction of carbohydrate and fatty acid oxidative processes in the aged. This research has impact on the mechanism underlying age-related changes in skeletal muscle activity, cardiac function and metabolism.

Methods Employed: As model systems, mitochondria isolated from mammalian cardiac muscle and insect flight muscle are employed.

Major Findings: A major locus of regulation in intermediary metabolism, and a problem of special concern in aging, is the interaction between the oxidation of carbohydrate and fat. This control is crucial, for during low levels of food intake and/or high work-loads the increased availability of fatty acids from reserve stores inhibits the oxidation of glucose in the heart and thus spares glucose for the brain, for which it is a mandatory fuel. The site of the regulation is the mitochondrial pyruvate dehydrogenase complex, which is inactivated during lipid oxidation, thus sparing pyruvate and, indirectly, glucose. Inactivation/ activation mechanisms for control of the activity of the dehydrogenase complex was shown to be mediated by covalent-linked phosphorylation/dephosphorylation reactions. Heart pyruvate dehydrogenase was found to be sensitive to the ratios of intramitochondrial NAD<sup>+</sup>/NADH, CoASH/acetylCoA, and ADP/ATP, such that an increase in each ratio gave a decreased degree of pyruvate dehydrogenase phosphorylation, and an increase in enzymatic activity.

The effect of fatty acid oxidation was to enhance phosphorylation of the enzyme by the pyruvate dehydrogenase kinase, favoring the inactivated form of the enzyme. Mitochondria from hearts of aged (24 months) rats showed a significantly lesser effect of fatty acid oxidation on pyruvate dehydrogenase interconversion than did mitochondria from mature (6 months) animals. Thus, glucose was not spared. This finding suggests that the aged, when subject to specific types of stress, can not frugally reserve glucose for vital functions, *e.g.* brain metabolism.

The significantly lesser effect of fatty acid oxidation on pyruvate dehydrogenase interconversion could reflect either an alteration in the pyruvate dehydrogenase interconversion enzymes or a lesser activity of fatty acid oxidation in heart mitochondria from senescent animals. The latter possibility was investigated first. It was found that the rate of oxygen consumption was diminished by about 30% in mitochondria from the senescent animals ( $p < 0.005$ ) with palmitoylcarnitine as substrate. This decrease was specific to fatty acid oxidation and was not seen with other NAD-linked substrates. It is not, therefore, a trivial artifact of mitochondrial preparation. In an attempt to

define more closely the locus of this age-linked decrement, the complex pathway of fatty acid oxidation to  $\text{CO}_2$  and water was dissected into several portions, by the use of appropriate substrates. In sequence, these portions are (1) carnitine: acylcarnitine exchange (entry of the acylcarnitine into the mitochondrion); (2) carnitine acyltransferase; (3)  $\beta$ -oxidation and (4) the tricarboxylate cycle. Whereas palmitoylcarnitine oxidation involves 1 through 4, acetylcarnitine oxidation involves only 1, 2 and 4 whereas octanoate oxidation involves only 3 and 4, plus (uniquely) acyl-CoA synthetase. By use of these substrates and others, it was established that there are several different sites of decrement in activity with senescence.

Measurement of the activities of the individual enzymes suspected from the reasoning above to be decreased with senescence gave significant decreases in octanoyl-CoA synthetase, carnitine acetyltransferase and  $\beta$ -hydroxyacyl-CoA dehydrogenase. The similar degree of loss of activity (*circa* 30%) raises the possibility of a co-ordinated control of the synthesis (or degradation) of these enzymes.

The fact that the oxidation of both species of acylcarnitine which were tested was diminished with senescence and the normally rate-limiting character of mitochondrial transport processes, suggested that carnitine: acylcarnitine exchange across the mitochondrial membrane be measured directly. This was done and it was found that the process was less active in the mitochondria from senescent animals. However, further investigation revealed that this reflected a decreased pool of mitochondrial carnitine and an unchanged first-order rate constant of exchange rather than any change in the carrier molecule *per se*. In a very recent study a diminished whole-heart content of carnitine in senescence was reported, so that the mitochondrial preparations appear to give an accurate picture of the *in vivo* situation. These mitochondrial findings, *i.e.* the description of carnitine efflux from the mitochondria as a first-order process, the demonstration of an unchanged first-order rate constant and the demonstration of a diminished mitochondrial carnitine pool with senescence, all define a decreased rate of carnitine:acylcarnitine exchange in the senescent heart, with implications for all metabolic pathways involving that exchange.

Last year we reported that the NAD-linked isocitrate dehydrogenase (IDH) was the rate-limiting enzyme of the tricarboxylate (Krebs) cycle, the major bioenergetic pathway. We have continued and completed studies on the regulation of this enzyme from mitochondria of blowfly flight muscle using a solubilized and partially purified preparation of the enzyme (8- to 10-fold, 25-30% recovery).  $2+\text{Mg}$ , Isocitrate, ADP, inorganic phosphate (Pi), citrate, fluorocitrate,  $2+\text{Mn}$  and increasing pH have been shown to be effectors of the NAD-IDH. These effectors activate the enzyme by increasing the affinity of the enzyme for isocitrate (decreasing the apparent Michaelis constant, or  $K'_m$  while, with the exception of increasing pH, not affecting the apparent maximum velocity ( $V'_m$ ) of the reaction. Increasing the pH caused both a decrease in  $K'_m$  and an increase in the  $V'_m$  of the reaction. In the presence of only isocitrate and  $2+\text{Mg}$ , at pH 7.3, the  $K'_m$  for isocitrate was  $2.6 \pm 0.05$  mM. Addition of 1 mM ADP reduced the  $K'_m$  to  $0.48 \pm 0.09$  mM; 1 mM citrate reduced

the  $K'_m$  to  $2.0 \pm 0.2$  mM; addition of 1 mM ADP + 10 mM Pi reduced the  $K'_m$  to 0.43 mM; 1 mM ADP + 1 mM citrate reduced  $K'_m$  to  $0.31 \pm 0.04$  mM; 1 mM citrate + 10 mM Pi, the  $K'_m$  was 1.5 mM; 1 mM ADP + 10 mM Pi + 1 mM citrate, the  $K'_m$  was  $0.19 \pm 0.04$  mM; and decreasing the  $Mg^{2+}$  concentration to 1 mM from 2.7 mM, in the absence of the other effectors, caused an increase in the  $K'_m$  to 3.9 mM.

The interaction of more than one effector with the enzyme is complex. Thus, increasing the concentration of substrate caused a decrease in the  $K'_m$  of the enzyme for ADP, citrate and  $Mg^{2+}$ ; increasing the ADP concentration caused a decrease in  $K'_m$  for isocitrate, citrate and  $Mg^{2+}$ . Addition of limiting concentrations of two or more effectors resulted in activation of the enzyme to a greater extent than predicted by simple additivity of the effects. Less fluorocitrate is required for activation of the enzyme than citrate, i.e. - the  $K'_m$  for fluorocitrate is nearly 3-fold less than that for citrate. Moreover, in the presence of a concentration of isocitrate that gave an enzyme activity of 10% of the maximum, citrate or phosphate caused nearly a 6-fold increase in activity at pH 7.7 but only a 1.5-fold increase at pH 7.3. ADP, on the other hand, stimulated the enzyme equally well, to about 12-fold, at both pH values and under the same conditions.

Calcium, ATP and decreasing pH (increased concentration of  $H^+$ ) have been shown to inhibit the enzyme. ATP and calcium increased the  $K'_m$  of the enzyme for isocitrate without affecting the  $V'_{max}$  of the reaction while decreasing the pH caused a decrease in the  $K'_m$  for isocitrate while decreasing the  $V'_{max}$  of the reaction.

Calcium, added as a  $CaCl_2$ /EGTA mixture of nominally equal molarity decreased the  $K'_m$  for isocitrate 2- to 3-fold. Such inhibition was also seen in the presence of ADP, Pi, citrate, fluorocitrate, ATP or at reduced pH. The concentration of free or unchelated calcium in such incubations was determined using a calcium electrode and was estimated to be in the range of 40-270  $\mu$ M, depending upon pH and concentration of effector present. The apparent inhibitor constant,  $K'_i$  for free calcium was estimated to be in the range of 160-220  $\mu$ M, again depending upon pH and effectors present.

ATP inhibited the NAD-IDH by increasing the  $K'_m$  for isocitrate by a factor of two in the absence of other effectors and by 4- to 10-fold in the presence of ADP, Pi and citrate. The extent of the increase in  $K'_m$  was markedly dependent upon the pH, being smaller at lower pH values. Under conditions where the concentration of total nucleotide (ADP + ATP) was held constant at 2 mM and in the presence of citrate and Pi, the ratio of ATP/ADP needed to give 50% inhibition of the enzyme was found to be 0.39 corresponding to an ATP concentration of 0.56 mM. In the absence of added effectors, and in the presence of a concentration of isocitrate sufficient to give an enzyme of 50% of maximum, the  $K'_i$  for ATP was 2.7 mM.

Addition of both ATP and calcium caused a nearly 3-fold increase in the  $K'_m$  for isocitrate in the absence of ADP and nearly 2-fold increase in the presence of ADP. Under these conditions, the concentration of free-calcium was found to be in the range of 40-60  $\mu$ M.

Studies measuring the electrochemical gradient of  $H^+$  activity across the membrane of blowfly flight muscle mitochondria were completed. The magnitude of this gradient (180 mV) was shown to be insufficient for its participation as an intermediate between electron flow ( $\Delta E = 270$  mV per site) and ATP formation ( $\Delta G_p = 270$  mV per site), data being for resting mitochondrial metabolism. However, if the chemiosmotic theory of energy transduction is modified so that 3  $H^+$  ions (rather than 2) are extended per site of the respiratory chain, then the proton gradient does indeed become a plausible intermediate. Direct measurements of  $H^+/O$  ratios in other laboratories and contemporary with the blowfly work are also consistent with 3  $H^+$  per site. Resting respiration (absence of added ADP) is a near-equilibrium situation and the correspondence of results obtained with the fly and those obtained with mammalian systems is to be expected. However, addition of ADP generates a non-equilibrium state and here the magnitude of the proton gradient reflects a resultant between the rate of generation, by electron transport, and dissipation, by phosphorylation of ADP. In this system, fly mitochondria behave very differently from those studied from mammals, because of the striking effect of ADP in activating isocitrate dehydrogenase. These experiments direct attention to the interaction of control at the respiratory chain and dehydrogenase levels, which is there in the mammal, but not so clear.

Significance to Biomedical Research and to the Program of the Institute: These studies define the mechanisms whereby age-dependent perturbation in physiological control systems may lead to the inability of the aged organism to maintain homeostasis. This fundamental information is needed for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities.

Proposed Course of the Project: Major effort will be put into further studies on carbohydrate:fatty acid competition for oxidation in heart, as the control is an important one and one which functions less well in the senescent animal. The idea is to find out to what extent the inactivation of pyruvate dehydrogenase by phosphorylation, as described above, is responsible for the lowered flux through the pyruvate dehydrogenase reaction, and to what extent end-product inhibition of that portion of the enzyme which has not been phosphorylated is involved.

Experimental portions of our studies using the unique properties of mitochondria from insect flight muscle have been completed.

Publications:

Hansford, R. G.: Studies on the effects of coenzyme A-SH:acetyl-coenzyme A, nicotinamide adenine dinucleotide and adenosine diphosphate: adenosine triphosphate ratios on the interconversion of active and inactive pyruvate dehydrogenase in isolated rat heart mitochondria. J. Biol. Chem. 251: 5483-5489, 1976.

Hansford, R. G.: Studies on the inactivation of pyruvate dehydrogenase by palmitoylcarnitine oxidation in isolated rat heart mitochondria. J. Biol. Chem. 252: 1552-1560, 1977.

Hansford, R. G.: A comparison of energy-yielding reactions in the flight muscle of young adult and senescent blowflies. Comp. Biochem. Physiol., in press.

Hansford, R. G.: Lipid oxidation by heart mitochondria from young adult and senescent rats. Biochem. J., in press.

Hansford, R. G. and Johnson, R. N.: Some aspects of the oxidation of pyruvate and palmitoylcarnitine by moth (*Manduca sexta*) flight muscle mitochondria. Comp. Biochem. Physiol. 55B: 543-551, 1976.

Johnson, R. N. and Hansford, R. G.: The nature of controlled respiration and its relationship to protonmotive force and proton conductance in blowfly flight muscle mitochondria. Biochem. J. 164: 305-322.

Sacktor, B.: Biochemical adaptations for flight in the insect. Biochemical Society Symposia, 41: 111-131, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00044-04
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PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effects of Metals and Proteins on Nucleic Acids, Information Transfer, and Aging.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	J. Butzow	Commissioned Officer	LMA NIA
	P. Clark	Research Chemist	LMA NIA
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COOPERATING UNITS (if any)

F. Y. H. Wu, Biophysicist, Albert Einstein College of Medicine, New York

LAP/BRANCH

Gerontology Research Center, Laboratory of Molecular Aging

SECTION

Molecular Chemistry

INSTITUTE AND LOCATION

NIA, NIH, Baltimore City Hospitals, Baltimore, Maryland

TOTAL MAN-YEARS:

6.72

PROFESSIONAL:

5.72

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project focuses on the interaction of molecules concerned with genetic information transfer. A primary objective is to determine under what conditions metal ions are essential for information transfer, and under what conditions they produce errors in the information and may thus contribute to biological aging. Topics of interest are: (1) The effects of metal ions on the structure of nucleoproteins and chromatin; (2) Age related changes in chromatin structure; (3) Aging of ribosomes; (4) Degradation of polynucleotides and dephosphorylation of nucleotides by metal ions; (5) Crosslinking of nucleic acid strands by metal ions; (6) The effects of metal ions on RNA polymerase; (7) Metal ions and cellular aging.



## Project Description:

Objectives: (1) To study effects of metal ions on the structures involved in the replication, transcription, and translation of genetic information transfer, (2) to determine how errors can be introduced into genetic information transfer by metal ions, and how these errors may affect aging, (3) to understand the interactions of proteins and nucleic acids and their mediation by metal ions, (4) to understand the role of metal ions in toxicity and aging, (5) to understand the participation of metal ions in the biological activities of nucleic acids and nucleoproteins, (6) to elucidate structural and functional changes in genetic information transfer that accompany aging.

Methods Employed: (1) The interaction of metal ions, proteins, and nucleic acids are studied by optical rotatory dispersion, circular dichroism, and spectrophotometry to determine conformational changes, and by infrared, nuclear magnetic resonance, and electron spin resonance techniques to determine interaction sites. (2) Chromatin structure is analyzed by means of model systems to determine which structural features are responsible for its function. (3) Physical chemical techniques are employed to study age changes in chromatin. (4) Effects of metal ions on nucleic acids and nucleoprotein are studied to determine under what conditions they serve an essential function in information transfer, and under what conditions they induce errors in information content. (5) The effect of metal ions on the enzymes responsible for genetic information transfer are studied. (6) The mechanisms by which enzymes and metal ions synthesize and degrade nucleic acids are elucidated.

Major Findings: A. Age related changes in chromatin structure. Chromatin, the repository of genetic information in the cell nucleus, is a complex of DNA and protein molecules. We have postulated that any age changes in the structure of chromatin should be reflected in different accessibility of the chromatin DNA to degrading enzymes. We therefore selected micrococcal nuclease to probe the chromatin structure. We have already reported preliminary results indicating that DNA from old rat liver chromatin is more rapidly hydrolyzed than DNA from young rat liver. This finding has now been confirmed by a comparison of 5 young (8-9 mo.) and 7 old (22-27 mo.) rats. The amount of DNA degraded after 180 min. averaged  $4.07 \times 10^{-5}$  mM for the young, and  $6.22 \times 10^{-5}$  mM for the old, with  $p < 0.002$ . The amount of DNA degraded after 210 min averaged  $4.76 \times 10^{-5}$  and  $7.09 \times 10^{-5}$  mM for young and old, respectively, with  $p < 0.001$ . Thus the rate of degradation of DNA increases with age, when reaction rates are considered. Nevertheless, preliminary results indicate that at the end point of the degradation process more DNA has been hydrolyzed in young than in old samples. The difference is small and requires corroboration.

The difference in DNA degradation rate was observed only when chromatin was obtained from freshly isolated nuclei. If the nuclei were allowed to stand for only two hours, and the chromatin from such nuclei used for the experiments with micrococcal nuclease, the age difference was wiped out, and in fact the degradation rate was generally that previously observed with old chromatin.

This observation leads to the likelihood that age differences are due to differences in the association of the molecules in the chromatin, rather than differences in the molecules themselves. The phenomenon is reminiscent of an earlier observation in our laboratory that a chromatographic profile of histones from old rat liver chromatin differs from such a profile from young chromatin, but chromatin from young nuclei isolated in the absence of divalent metals produces a profile similar to that obtained from old chromatin. The profile thus reflects ease of detachment, and not composition. In the absence of the metals the ordered association of the macromolecules appears to be affected in a manner similar to that produced by aging.

Changes in structure of anisotropic substances such as chromatin and chromatin constituents often lead to changes in circular dichroism, which in fact measures anisotropy. We therefore compared circular dichroism spectra of chromatin from young and old rat livers. Since the chromatin solutions are turbid, their circular dichroism spectra are somewhat variable; nevertheless, a small bathochromic shift is usually observed in old, compared with young, chromatin. Solutions from old chromatin exhibit greater variability than solutions from young chromatin. We thus have two structural probes that indicate changes in the way in which macromolecules are associated in young and old chromatin.

B. Metal ions and the compaction of DNA-polypeptide complexes. The complexing of basic proteins to DNA in the eukaryotic cell nucleus produces a highly compacted chromatin structure that is influenced by divalent metal ions. Age changes in chromatin have been correlated with changes produced by metal ions. The basic proteins contain much lysine; polylysine and lysine-containing synthetic polypeptides complexed to DNA can be used as models for the interactions in chromatin, so that the essential features of these interactions can be understood. Polylysine and poly(Lys<sub>70</sub>Ala<sub>30</sub>) lead to one kind of compaction of DNA characterized by highly negative circular dichroism (CD) which we have called the  $\psi(-)$  structure, whereas poly(Lys<sub>50</sub>Ala<sub>50</sub>) and poly(Lys<sub>30</sub>Ala<sub>70</sub>) lead to a different type of compaction characterized by a highly positive CD, which we have called the  $\psi(+)$  structure. Histones H-1 and H-4 complexes of DNA have  $\psi(-)$  and  $\psi(+)$  structures, respectively.

The interpolation of neutral amino acids between lysine residues can convert a  $\psi(-)$  structure to  $\psi(+)$ , as a comparison of solutions of DNA polylysine and DNA-poly(Lys<sub>50</sub>Ala<sub>50</sub>) having the same number of lysine residues/DNA demonstrates: the former is  $\psi(-)$ , the latter  $\psi(+)$ . Phosphate binding metal ions (e.g. Mg<sup>2+</sup>) enhance the CD effect not only of the  $\psi(-)$  structures, as previously shown, but also of the  $\psi(+)$  structures. We previously reported that some base binding metal ions and complexes, e.g. Cu<sup>2+</sup> and cis[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>] can reversibly convert the DNA-polylysine  $\psi(-)$  to  $\psi(+)$ . These metals affect other  $\psi(-)$  structures in the same manner. Other base binding metal ions (e.g. Ag<sup>+</sup>) have the reverse effect;  $\psi(+)$  is converted to  $\psi(-)$ . The varied influences of the metals is illustrated by the fact that cis[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>] converts  $\psi(-)$  to  $\psi(+)$  and enhances the CD of  $\psi(+)$  structures; Ag<sup>+</sup> converts  $\psi(+)$  to  $\psi(-)$  and enhances the CD of

$\psi(-)$  structures;  $\text{trans}[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$  has an intermediate effect; it diminishes the  $\psi(-)$  CD without conversion to  $\psi(+)$ . The differences between the cis and trans isomers of the Pt complex are noteworthy in view of the activity of the cis but not the trans isomer as an antitumor agent.

Both the conversion of  $\psi(+)$  to  $\psi(-)$  and the enhancement of the CD characteristics of  $\psi(-)$  structures by  $\text{Ag}^+$  ions can be reversed completely by complexing  $\text{Ag}^+$  with  $\text{Cl}^-$  or  $\text{CN}^-$  ions. The effects of metal ions are generally reversible in this way. Reactions of DNA-polypeptide systems with metal ions are therefore versatile tools for controlling at will the way in which DNA molecules are compacted.

The effect of the metals is to reorganize an already organized structure. Neither  $\psi(+)$  nor  $\psi(-)$  structures can be produced by the addition of a Pt complex or  $\text{Ag}^+$  ion to solutions of DNA and polypeptides that were formed by direct mixing. Only DNA polypeptide complexes produced by gradient dialysis and thus already poised in an ordered structure are susceptible to the metal ion effects. The  $\psi$  structure is not produced by the addition of polypeptides to metal complexes of DNA. We conclude that polypeptides under certain conditions can organize the DNA molecules into a rigid matrix whose exact orientation depends on the nature of the polypeptide, and which can be perturbed by metals.

The existence of such a matrix is further demonstrated by the fact that radioactive DNA does not exchange with any DNA present in a gradient dialyzed solution, even if the DNA is present in excess. The matrix theory also explains the following phenomena: Neither  $\text{Cu}^{2+}$  nor  $\text{cis}[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$  convert  $\psi(-)$  of 1:0.8 DNA-polylysine to  $\psi(+)$ . The addition of sufficient DNA to produce a 1:0.5 ratio, which would be susceptible to the conversion if all the DNA had been present prior to gradient dialysis, still does not make the conversion possible. These experiments can be explained only by the presence of a matrix that can accommodate varying quantities of DNA.

The degree of order of the DNA matrix, as measured by CD, though enhanced by phosphate binding metals such as  $\text{Mg}^{2+}$  at moderate concentrations, can be distorted by heating in the presence of these concentrations of metal or by the addition of excess metal. Such distortions arise from the crosslinking of DNA molecules by polypeptides. As is frequently the case, metals can have opposite effects on structures when conditions are varied.

C. Metal ions and errors in transcription. The genetic information from the DNA in chromatin is transcribed into RNA in the presence of the enzyme RNA polymerase. The enzyme from E. coli requires activation by divalent metal ions. In the presence of  $\text{Mg}^{2+}$  it is believed that only the "correct" incorporation of ribonucleotide into RNA takes place; Hurwitz et al. have shown that  $\text{Mn}^{2+}$  induces the "incorrect" incorporation of deoxynucleotide along with the desired ribonucleotide. Such misincorporation of deoxynucleotide into RNA through the agency of metal ions could lead to the kind of error in the structure of an information molecule that could produce deleterious changes in an organism.

There has been no previous direct comparison of the effect of various metal ions on the incorporation of ribonucleotide and deoxynucleotide. The effect of metals on ribonucleotide incorporation itself has not been studied with enzyme purified by present methods. We therefore first studied the metal concentration dependence of the enzyme activity, and found optima (for ribonucleotide incorporation) at a relatively low concentration, 0.25 mM, with  $\text{Co}^{2+}$  and  $\text{Mn}^{2+}$ ; activation by  $\text{Mg}^{2+}$  was maximal at much higher concentration, 25 mM or higher. Other metals did not activate the enzyme.

We confirmed that  $\text{Mn}^{2+}$  causes the error incorporation of deoxynucleotides into RNA.  $\text{Co}^{2+}$ , like  $\text{Mg}^{2+}$  causes the incorporation of ribonucleotides only. Thus, of the three activating metals,  $\text{Mg}^{2+}$  and  $\text{Co}^{2+}$ , but not  $\text{Mn}^{2+}$ , appear capable of effectively differentiating between ribonucleotides and deoxynucleotides.

We postulated that  $\text{Co}^{2+}$  and  $\text{Mg}^{2+}$  bound enzymes have conformations sensitive to the difference between ribo- and deoxynucleotides, and that  $\text{Mn}^{2+}$  bound enzyme has a different conformation that lacks such sensitivity. We therefore carried out studies to determine whether we could in fact observe conformational differences between the various metal-enzyme complexes. Circular dichroism (CD) as well as optical rotatory dispersion (ORD) studies at room temperature revealed no differences between  $\text{Mn}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Co}^{2+}$  bound enzymes. These techniques would not detect localized conformational changes that do not affect the whole molecule. However, uv and ORD melting curves of the  $\text{Co}^{2+}$  and  $\text{Mn}^{2+}$  enzymes exhibited denaturing transitions at lower temperatures than the  $\text{Mg}^{2+}$  enzyme. The transitions were accompanied by precipitation, indicating that aggregation occurs during these transitions. Comparisons of the melting curves were made at metal ion concentrations reflecting optimal activating ability. However, the dichotomy between  $\text{Co}^{2+}$  and  $\text{Mg}^{2+}$  on the one hand and  $\text{Mn}^{2+}$  on the other remained constant during variations in the transition temperature produced by altering the enzyme concentrations. We do not know at present whether the correlation between melting transitions and sensitivity to correct substrate reflects a causal relationship or is merely coincidental and hope that further studies will clarify the matter.

D. Metal Ion Dephosphorylation of Nucleotides. Among the potentially most destructive effects of metal ions on nucleic acids is their ability to degrade RNA (but not DNA). Certain metal ions are exceedingly effective in this degradation; among them are  $\text{Zn}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Ce}^{3+}$ . In the case of  $\text{Zn}^{2+}$  the degradation stops at the level of the mononucleotide; the latter is not further dephosphorylated to a nucleotide. Such dephosphorylation does occur with  $\text{Pb}^{2+}$  and  $\text{Ce}^{3+}$ . We therefore asked why  $\text{Zn}^{2+}$  ions can act on internucleotide bonds but not on the single phosphate on a mononucleotide, and why in contrast  $\text{Pb}^{2+}$  and  $\text{Ce}^{3+}$  can go on to remove this last phosphate. To try to answer this question we have compared the reactivity of a variety of nucleotides with these metals over a range of conditions, and correlated this reactivity with physical parameters of the metal complexes, such as circular dichroism (CD), solubility at room temperature and reaction temperature, titration behavior, and hydrolytic tendency.

We have previously noted for  $Pb^{2+}$ , and can now confirm for  $Ce^{3+}$ , that the most reactive adenine nucleotides are 2' and 3'AMP, which have OH groups on the ribose adjacent to the site of the phosphate to be cleaved. 5'dAMP has intermediate reactivity and 5'AMP is always least reactive. The catalytic activity of the metal ions depends on their ability to polarize electrons in their direction and therefore away from the bond to be cleaved; the hydroxyl groups adjacent to the cleavage site presumably aid the metal by simultaneously withdrawing electrons from the sugar ring.

Of the three metals under consideration,  $Ce^{3+}$  is the most active dephosphorylating agent and  $Zn^{2+}$  is least active, with  $Pb^{2+}$  intermediate. The great difference between  $Ce^{3+}$  and  $Pb^{2+}$  in reactivity toward adenine nucleotides correlates very well with the CD spectra of the complexes. Nonreactive zinc complexes have very intense CD spectra, while highly reactive cerium complexes have virtually no CD spectrum. The intense CD spectra of the  $Zn^{2+}$  complexes indicate a high degree of nucleotide stacking, and we conclude that this stacking protects the phosphate groups and prevents dephosphorylation. In contrast, the lack of stacking of  $Ce^{3+}$  complexes provides no inhibition for the reaction.

$Pb^{2+}$  produces extensive dephosphorylation, although less than  $Ce^{3+}$ ; nevertheless the  $Pb^{2+}$  complexes have significant CD spectra. To understand the differences between the  $Pb^{2+}$  and  $Zn^{2+}$  complexes requires a more thorough analysis in which pH is taken into consideration. The pH profile for  $Pb(II)$  3'AMP, for example shows that the CD effect is maximal at low pH and almost obliterated at pH 7. Above pH 8 extensive hydrolysis of Pb occurs. There is thus a window around pH 7 in which neither extensive stacking nor metal hydrolysis limit the reactivity of the phosphate group, permitting dephosphorylation. pH 7 is in fact the optimal pH for the reaction, which is also favored at this pH by solubility considerations. Similar analyses have been made of other  $Pb(II)$  nucleotide complexes and account for their reactivity. Such analyses of Zn complexes show that there is no window for the dephosphorylation reaction. The ability of metal ions to promote dephosphorylation therefore depends on the interplay of many factors, including the presence of a hydroxyl group adjacent to the phosphate to be cleaved, lack of nucleotide stacking, metal hydrolysis, and solubility of the complex. The ability of metal ions to dephosphorylate nucleotides obviously can be a factor in their toxicity.

E. Mechanism of DNA cleavage by deoxyribonucleases. It is theoretically possible for enzymes to cleave double stranded DNA in one of the following ways: (1) single chain cleavage; i.e., enzyme hits only one DNA chain at each cleavage site, (2) double chain cleavage, i.e., the enzyme hits both chains simultaneously at the site of a complementary base pair, (3) double chain cleavage with one unpaired base at each end of a fragment, and (4) double chain cleavage, with two or more unpaired bases at each end of the fragment.

A method has been devised to distinguish between these mechanisms by fitting the results of base analyses of fragments produced by the enzymatic hydrolysis of DNA to mathematical analyses that predict the base content of the fragments for each mechanism. By application of this method it has been found that

DNase I is cleaved according to mechanism 1, regardless of which activating metal is used. For DNase II the method rules out mechanisms 1 and 4, but cannot quite distinguish between mechanisms 2 and 3.

F. Metal ions in early and late passage cells. The previously observed changes in the concentrations of various metal ions between early and late passage of WI-38 cells have been corroborated with cells obtained from E. L. Schneider. As has been noted, the concentrations of many metal ions per cell, i.e. per DNA content, increases, although such increases cannot be substantiated when concentrations are calculated as concentration per weight of cell; this weight of course also increases with age. The measured concentration changes per DNA content are, however, most significant to us in terms of our interest in the effect of metal ions on genetic information transfer.

Significance to Biomedical Research and to the Program of the Institute: The studies on the age changes in chromatin and metal contents of cells are of obvious relevance. The participation of metal ions in every aspect of genetic information transfer and the deleterious effects on this transfer caused by undesired metal ions or essential metal ions in undesired concentrations make the study of metal ion interactions with nucleic acids of major importance. The possible relationship between aluminum accumulation and Alzheimer's disease and the discovery that the aluminum is bound to chromatin have emphasized the importance of studies on metal interaction with nucleic acids and chromatin. An understanding of the structure and function of chromatin (and therefore protein - DNA interaction), ribosomes, the nucleic acid polymerases, etc., is essential to an understanding of cellular aging. We are particularly interested in studies that show how information transfer can go wrong. Metal ions are presumably not responsible for the primary events that cause aging but we believe that they may be important factors in determining individual and geographic differences in the aging process.

Proposed Course of Project: We plan to extend our studies of nucleic acid-polypeptide interaction to include aluminum, and to study the effect of aluminum on RNA polymerase; we have begun a collaborative effort with D. Crapper of the University of Toronto on the interaction of DNA with aluminum and other studies to elucidate the possible relationship between aluminum and Alzheimer's disease.

We shall continue our studies on the effects of metal ions on DNA-protein interaction, later apply them to histone-DNA interaction, and eventually we hope to understand the role of metals in the make-up of chromatin and the cell nucleus. At the same time we shall continue to study age changes of chromatin and perhaps the intact nucleus by chemical and physical probes. We also intend to continue our studies of the effects of metal ions on nucleic acids in vitro.

We wish to exploit the discovery that  $\text{Co}^{2+}$  is like  $\text{Mg}^{2+}$  in differentiating between the correct and incorrect substrate for RNA polymerase, and therefore

unlike  $Mn^{2+}$  which does not differentiate and causes error. Since both  $Co^{2+}$  and  $Mn^{2+}$  (but not  $Mg^{2+}$ ) are paramagnetic, it is possible to use paramagnetic effects in nuclear magnetic resonance to probe the enzyme-metal-substrate complex with one metal that does and one that doesn't differentiate. As the Mildvan group at Fox Chase Cancer Institute is looking at the inorganic enzyme, we intend to study the cobalt enzyme with the collaboration of Don Hollis at Johns Hopkins Medical School.

We expect to begin a new project to test for age changes in the ribosome. It has been shown previously that high metal concentrations lead to the incorporation of wrong amino acids into protein, and that higher metal concentrations are required for this misincorporation with eukaryotic than with bacterial ribosomes. Thus it occurs to us that the efficacy of a ribosome can be tested by challenging it with increasing metal ion concentrations and determining at what concentration the ribosome can no longer maintain fidelity in protein synthesis. We expect that it may be easier to detect age changes in ribosomes if they have been stressed, and high metal ion concentration appears to be a convenient and logical way to apply the stress.

#### Publications:

Rifkind, J. M., Shin, Y. A., Heim, J. M. and Eichhorn, G. L.: Cooperative disordering of single-stranded polynucleotides through copper crosslinking. Biopolymers 15: 1879-1902, 1976.

Shin, Y. A. and Eichhorn, G. L.: Reversible change in  $\psi$  structure of DNA-poly(Lys) complexes induced by metal binding. Biopolymers 16: 225-230, 1977.

Butzow, J. J. and Eichhorn, G. L.: The presence of RNase II in high salt washed E. coli ribosomes: effect on circular dichroism of ribosomal complexes. Nucleic Acids Research 4: 867-876, 1977.

Eichhorn, G. L., Rifkind, J., Shin, Y. A., Butzow, J., Clark, P. and Froehlich, J.: Recent Studies on the Effects of Divalent Metal Ions on the Structure and Function of Nucleic Acids. In Pullman, B., and Goldblum, N. (Eds.): Metal-Ligand Interactions in Organic Chemistry and Biochemistry. Theory and Experiment. Holland, Reidel Publishing Co., 1977, 41-51.

Eichhorn, G. L.: Bioinorganic Chemistry. In McGraw-Hill Encyclopedia of Science and Technology, 4th Edition, in press.

Eichhorn, G. L., Rifkind, J. M. and Shin, Y. A.: The effect of copper on nucleic acid and nucleoprotein conformation. Adv. Chem., in press.





## Project Description:

Objectives: Ehrlich, of Salvarsan fame, recognized that "corpora non agunt nisi fixata." This maxim seems to hold even for macromolecules. The present compounds have been designed for (a) a study of possible anchoring points for macromolecules on cell surfaces with a view to determine eventually how the number of receptor sites differs with age or transformation and for (b) a study of methods to destroy at will the points where macromolecules are anchored.

Methods Employed: The study requires both chemical and biological methods. Methods for attachment of small molecular weight compounds to polysaccharides were investigated and the stability of the attachment measured under physiological conditions. These compounds were then tested on (a) mouse erythroleukemic cells which grow in suspension and thus are easily obtainable in bulk and (b) human fibroblast cells, which grow in monolayers and are thus obtainable only in smaller amounts.

Major Findings: Mercury-dextran. This compound is as toxic to cells in culture as are free mercury ions. Binding of mercury dextran to cells is time dependent, and the surface can be saturated; the presence of free sulfhydryl groups on the cell surface is therefore indicated. The number of the free sulfhydryl groups on the cell surface can be increased about ten times by treatment of cells with reagents which reduce disulfide groups into sulfhydryls. Such treatment makes the cell surface even more susceptible to the action of mercury dextran. Treatment of cells with a macromolecular reducing agent does not lead to the reduction of disulfide bonds; thus these bonds are probably located in inaccessible places.

Diazonium dextran. This reagent is also bound to cells, thus the surface of cells has exposed tyrosine and histidine residues. The reagent is toxic to cells.

Nucleic Acid Binding Dyes Attached to Dextran. The parent dyes have considerable toxicity; their attachment to dextran (a) decreases their toxicity and (b) strongly decreases their penetration into cells.

Oxygen activating dyes attached to dextran - Rose Bengal is toxic to cells only in the presence of light. This dye was attached to a basic dextran, a compound strongly absorbed by cells; the resulting macromolecule has higher phototoxicity than the parent dye.

Significance to Biomedical Research and Program of the Institute. Macromolecules differ profoundly from low molecular weight compounds in their biological potentials. The present studies aim to use these differences in the investigation of the cell surface, a cellular component which is supposed to reflect closely the state and age of the cell itself.

Proposed Course of Project. By directed synthesis and study of the basic biological effects of macromolecules it is hoped to gain knowledge necessary for the design of practically useful compounds; e.g. antiviral drugs and drugs that inhibit virus specific enzymes, reagents that react with the cell surface and specific groups on the cell surface, and reagents that can detect surface charge.

Publications

Blob, L. N., Vengris, V. E., Pitha, P. M. and Pitha, J.: Uptake and fate of water-soluble, nondegradable polymers with antiviral activity in cells and animals. J. Med. Chem. 20: 356-359, 1977.

Pitha, J.: Polymeric analogs of nucleic acids. Polymer 18, 425-430, 1977.

Pitha, J.: Fractionation of electroneutral polymer by gel electrophoresis in the presence of ionic detergent. Macromolecules 9: 771-773, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00047-07 LMA						
PERIOD COVERED July 1, 1976 to September 30, 1977								
TITLE OF PROJECT (80 characters or less) Relation of Structure and Function in Hemoglobin								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT								
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: J. M. Rifkind</td> <td style="width: 33%;">Research Chemist</td> <td style="width: 33%;">LMA NIA</td> </tr> <tr> <td>OTHER: K. Araki</td> <td>Visiting Fellow</td> <td>LMA NIA</td> </tr> </table>			PI: J. M. Rifkind	Research Chemist	LMA NIA	OTHER: K. Araki	Visiting Fellow	LMA NIA
PI: J. M. Rifkind	Research Chemist	LMA NIA						
OTHER: K. Araki	Visiting Fellow	LMA NIA						
COOPERATING UNITS (if any) K. Moffat, Professor of Biochemistry, Cornell University, Ithaca, N.Y.; J. Bonaventura, Professor of Biochemistry, Duke University, Beaufort, N.C.; F. Padilla, Chief of Hematology & Oncology, Little Rock Veterans Hospital,								
LAB/FRANCH Little Rock, AR. Gerontology Research Center, Laboratory of Molecular Aging								
SECTION Molecular Chemistry								
INSTITUTE AND LOCATION NIA, NIH, Baltimore City Hospitals, Baltimore, Maryland 21224								
TOTAL MANYEARS: 2.2	PROFESSIONAL: 1.7	OTHER: 0.5						
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<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to study the mechanisms involved in regulating the binding of oxygen to <u>hemoglobin</u> and the <u>transport of oxygen</u> to the tissues. The project also focuses on ways in which these functions are impaired and <u>change with age</u> . We have recently found that zinc dramatically increases the <u>oxygen affinity</u> of hemoglobin and that this effect is coupled to the interaction between hemoglobin and <u>2,3-diphosphoglyceric acid</u> (DPG), which is thought to play a major role in regulating the release of oxygen from hemoglobin. We have also studied the mechanisms involved in the <u>oxidation</u> of hemoglobin. Oxidation effects oxygen transport because it produces nonfunctional hemoglobin, which no longer binds oxygen. Recently we have extended these studies to include an investigation of the stability of the <u>erythrocyte</u> as measured by <u>osmotic fragility</u> , which can determine the functional lifespan of the hemoglobin, and, thereby, the level of hemoglobin maintained in circulation.								

## Project Description:

Objectives: (1) To study the binding of ligands to hemoglobin, and the role of the protein in controlling this function. (2) To study the mechanisms for maintaining hemoglobin in its functional form. (3) To study the mechanisms involved in regulating the transport of oxygen to the tissues.

Methods Employed: Various preparative procedures are used to purify hemoglobin and to separate various components of the erythrocyte. Visible, uv and atomic absorption spectroscopy, as well as gel electrophoresis is used to analyze for various erythrocyte components. The oxygenation and oxidation of hemoglobin is investigated under various conditions with and without the addition of various substances. Binding of metal ions and other small substances are studied by equilibrium dialysis. Electron spin resonance is used to observe paramagnetic Cu(II) bound protein and to follow the changes in paramagnetic species found as hemoglobin is oxidized. The extent of hemolysis and the rate of hemolysis of various samples of red cells are observed at various hypotonic salt concentrations.

Major Findings: A. The Location of Metal Binding Sites on Hemoglobin. We have been investigating the binding of Zn(II) and Cu(II) to hemoglobin, and their effect on the oxygenation and oxidation of hemoglobin, respectively. In order to understand these reactions it is necessary to determine the location of these metal ions on hemoglobin which contains four subunits and a total of 574 amino acid residues. We have been able to interest K. Moffat of Cornell University, an x-ray crystallographer, in this problem. We now have preliminary results which suggest a similar binding site for Cu(II) and Zn(II) in horse oxidized hemoglobin.

At the same time we have been investigating the metal ion reactions of various hemoglobin variants. We have recently been able to obtain from J. Bonaventura of Duke University and F. Padilla of Little Rock Veterans Hospital several abnormal human hemoglobins in which a single amino acid has been replaced by another amino acid with a very different metal ion affinity. Our studies on the oxygenation, oxidation, and metal ion affinities of these abnormal hemoglobins strongly suggest that the Zn(II) binding site associated with the oxygenation and the Cu(II) binding site associated with the oxidation both involve histidine  $\beta$ -143.

This amino acid is located some distance away from the heme at the interface between both  $\beta$ -chains, a region which is known to be conformationally altered by reactions taking place at the heme. The very different effects of binding Zn(II) and Cu(II) to this region of the molecule must be related to the known different oxidation potentials of these two metal ions.

B. Regulation of the Oxygen Affinity of Hemoglobin. 2,3-diphosphoglyceric acid (DPG), a major product of glycolysis within the erythrocyte, is thought to play a major role in regulating the physiological oxygen affinity of hemoglobin. This "cofactor" at least in part functions by preferentially binding to deoxyhemoglobin, thereby lowering the oxygen affinity of hemoglobin. The

dramatic zinc induced increase in the oxygen affinity of hemoglobin previously found in purified hemoglobin has been shown to be coupled to the interaction of 2,3-DPG and hemoglobin, with a decrease in the zinc effect as the level of 2,3-DPG is increased. Therefore, zinc may perhaps play a role in fine tuning the 2,3-DPG regulated oxygen affinity of hemoglobin. The mechanism for this interrelationship can be explained by the finding that histidine  $\beta$ -143 is involved in the binding of both zinc and 2,3-DPG. The rest of the binding site is however different in both cases. While 2,3-DPG binds preferentially to the deoxygenated conformation of hemoglobin, zinc binds preferentially to the oxygenated conformation of hemoglobin, and the environment of histidine  $\beta$ -143 is different in both of these conformations.

C. Oxidation of Hemoglobin by Copper. We have previously found that Cu(II) binds to hemoglobin and also oxidizes it producing nonfunctional hemoglobin which can no longer bind oxygen. However, a direct relationship between the binding and oxidation has not previously been demonstrated. We have now used electron spin resonance (ESR) to show that the copper is first bound to hemoglobin and only then oxidizes the heme. With certain hemoglobin species it has been possible to observe two different copper bound species both of which are formed prior to the oxidation, but only one of which proceeds to oxidize the heme.

The oxidation of hemoglobin, therefore, requires that copper be bound to a very specific hemoglobin site, which we have shown to be located some distance from the heme (see above). An understanding of the transfer of electrons between Cu(II) bound to this site and the Fe(II) of the heme should thus be of value not only in understanding the oxidation of hemoglobin, but also with respect to the very important electron transport processes involving other proteins.

D. The Effect of Age on the Fragility of Red Cells. We have previously limited our studies to those reactions of hemoglobin which can influence the transport of oxygen, and have neglected the requirement for the hemoglobin to be contained within an intact functioning erythrocyte in order to transport oxygen. As a measure of the stability of the erythrocyte we have now measured the range of hypotonic salt concentrations necessary to hemolyze the red blood cells. This distribution of osmotic fragilities can depend on the intracellular composition as well as the properties of the cell membrane itself, and is therefore a sensitive probe of erythrocyte alterations, which can take place both as the cells age and/or the individual ages. We find that the erythrocytes of older individuals are slightly more fragile ( $P < 0.05$ ) with a wider and more asymmetric distribution of fragilities ( $P < 0.001$ ). We furthermore find that, even though the cells of older individuals are more fragile, the rate of hemolysis is slower for older individuals ( $P < 0.005$ ).

E. The Age Distribution of Red Cells and the Fragility. The human erythrocyte has a lifespan of ~ 120 days, during which time numerous changes are known to take place. It is generally assumed that the fragility of the red cell increases as the cell ages. The cell density and the cellular activity of

glutamic oxalacetic transaminase (GOT) have been shown to change as the cells age and have frequently been used as standards for the aging of erythrocytes. We find that the mean fragility of the most dense and least dense fractions of cells is almost identical. We furthermore find that the highest GOT levels are found in cells of intermediate fragility. There is therefore no simple correlation between the age distribution and the fragility distribution, although the GOT results perhaps suggest that as the cells age the fragility distribution broadens without significantly shifting.

Significance to Biomedical Research and Program of the Institute. The physiological role of hemoglobin is to transport oxygen from the lungs to the cells. The efficient uptake and release of oxygen requires cooperative oxygen binding and the proper regulation of the oxygen affinity. It is also necessary to maintain the integrity of the erythrocyte and to limit the oxidation of hemoglobin in order to maintain an adequate concentration of functional hemoglobin in circulation. These studies thus help to elucidate a vital function of organisms. The aging process can involve changes in the ability of the organism to transport oxygen to certain tissues.

Proposed Course of the Project: (1) We plan to study the factors which determine the fragility of red cells in order to explain the origin of the observed fragility changes in older individuals and the relationship between the cell age distribution and the fragility distribution. (2) We plan to study the oxygenation of blood from individuals of various ages and try to correlate any observed changes with alterations in the erythrocyte composition and/or the hemoglobin molecule. (3) We plan to investigate the mechanism for the transfer of electrons between Cu(II) bound to hemoglobin at a site removed from the heme and the iron of the heme. Various techniques will be used to detect possible intermediates in this reaction. (4) We plan to investigate the possible physiological significance of the reported zinc induced increase in the oxygen affinity of hemoglobin.

#### Publications:

Rifkind, J. M., Lauer, L. D., Chiang, S. C. and Li, N. C.: Copper and the oxidation of hemoglobin: a comparison of horse and human hemoglobins. Biochemistry 15: 5337-5343, 1976.

Rifkind, J. M. and Heim, J. M.: The interaction of zinc with hemoglobin: binding of zinc and the oxygen affinity. Biochemistry, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00048-03 LMA
PERIOD COVERED July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (80 characters or less)  Mechanisms of Metal Ion Transport Across Cellular Membranes		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: J. P. Froehlich OTHER: E. Lakatta  R. W. Albers A. S. Hobbs	Medical Officer Chief, Cardiovascular Section Chief, Neurochemistry Section Staff Fellow	LMA NIA  CVS NIA  LNC NINDCDS LNC NINDCDS
COOPERATING UNITS (if any)  A. Schwartz, Professor and Chairman, Department of Pharmacology, University of Cincinnati; M. Sumida, Postdoctoral Fellow, University of Cincinnati		
LAB/BRANCH Gerontology Research Center, Laboratory of Molecular Aging		
SECTION Molecular Chemistry		
INSTITUTE AND LOCATION NIA, NIH, Baltimore City Hospitals, Baltimore, Maryland 21224		
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<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The enzymatic reactions involved in <u>Ca<sup>2+</sup> transport</u> in muscle and <u>Na<sup>+</sup> plus K<sup>+</sup> transport</u> in electric organ were investigated by <u>rapid chemical quenching</u> and <u>P<sup>31</sup> nuclear magnetic resonance spectroscopy</u> . Fast kinetic measurements of the ATPase reactions linked to Na <sup>+</sup> and K <sup>+</sup> transport have demonstrated unusual kinetic effects which suggest that the enzyme may have some "memory" of previous events. These effects have been interpreted as evidence that phosphoryl group transfer occurs at kinetically distinct but coupled catalytic sites in accord with the dimer <u>model</u> for transport. Comparison of phosphoprotein formation in sarcoplasmic reticulum preparations from <u>cardiac</u> and <u>skeletal muscle</u> have shown the rates to be nearly identical implying that the slower rate of <u>relaxation</u> in cardiac muscle arises from a lower density of transport sites rather than a reduced rate of turnover. P <sup>31</sup> NMR studies of sarcoplasmic reticulum have confirmed the existence of covalently bound phosphorous in membranes which are transport-viable. Similarities in the resonance spectra obtained with ATP or inorganic phosphate are consistent with kinetic observations showing the transport-linked catalytic pathways to be fully reversible.		

## Project Description:

Objectives: (1) To understand the relationship between active transport and the enzymatic reaction linked to transport; (2) to understand how cation transport systems convert chemical energy to osmotic work; (3) to understand how aging affects energy utilization during transport.

Methods Employed: The effects of metal ion activators of the enzymatic partial reactions catalyzed by  $\text{Ca}^{2+}$  and  $\text{Na}^{+}$  plus  $\text{K}^{+}$  ATPase have been investigated by a rapid mixing technique. The method permits resolution of rapid ( $t_{1/2} \geq .005$  seconds) enzymatic reactions having chemically stable intermediates or products. Fourier transform  $\text{P}^{31}$  nuclear magnetic resonance spectroscopy has been used to detect the presence of covalently bound phosphorus in sarcoplasmic reticulum membranes.

Major Findings: A. Kinetics of phosphoprotein formation in sarcoplasmic reticulum from cardiac muscle. Rapid chemical quenching was used to compare the initial time course of phosphorylation in skeletal and cardiac muscle sarcoplasmic reticulum preparations. Both preparations exhibit a rapid initial phase of labelling which exceeds the steady state level by approximately 20%. The rates of decay of the phosphoprotein overshoot in both preparations were similar implying that the rates of dephosphorylation are similar. The presence of virtually identical phosphorylation kinetics in these preparations suggests that the slower rate of relaxation observed in cardiac muscle may be due to a lower density of transport sites rather than to a slower rate of turnover.

B. Kinetics of  $(\text{Na}^{+} + \text{K}^{+})$ -dependent ATPase. The kinetics of phosphorylation and inorganic phosphate production depended on whether  $\text{K}^{+}$  was present with  $\text{Na}^{+}$  before addition of ATP or added with ATP. The difference in kinetic behavior resulting from the different preincubation conditions lasted for several hundred milliseconds suggesting that either the enzyme had some "memory" of previous events or that a fraction of the enzyme was turning over very slowly. Dephosphorylation of the enzyme initiated by addition of excess unlabelled substrate led to the demonstration of a slow component which did not appear to be part of the major catalytic pathway. The presence of a slowly decaying component suggests that phosphorylation can arise from kinetically distinct sites which may correspond to the separate halves of an enzyme dimer. At low ATP concentrations the kinetic coupling between sites may be weak so that they turn over almost entirely independent of one another.

C. Kinetics of  $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -dependent ATPase. Slowing of the rate of phosphorylation was observed following preincubation with high concentrations of  $\text{Mg}^{2+}$  or inorganic phosphate. The possibility that these species might competitively block the binding of  $\text{Ca}^{2+}$  and ATP was eliminated by rapidly diluting the contents of the enzyme syringe 20-fold at the time of addition of  $\text{Ca}^{2+}$  and ATP. The slowing of phosphorylation which follows preincubation with  $\text{Mg}^{2+}$  or inorganic phosphate implies that these products are able to stabilize a conformation which undergoes a slow transformation to an



intermediate which binds  $\text{Ca}^{2+}$  with high affinity. A slow transformation between product and substrate stabilized conformers thus constitutes the rate-limiting step in the overall reaction.

D.  $\text{P}^{31}$  NMR studies of phosphorylation group transfer in sarcoplasmic reticulum membranes. Transfer of the terminal phosphoryl group of ATP to membrane protein in the presence of  $\text{Ca}^{2+}$  results in the formation of phosphoenzyme which is stable under acidic (denaturing) conditions. Using  $\text{P}^{31}$  NMR spectroscopy it is possible to observe the phosphoenzyme under conditions in which the sarcoplasmic reticulum remains transport-viable. The resonance spectrum consists of one low-field and two high-field bands; the large shift in the high-field peaks (~ 12 ppm) suggests that the phosphorus is in a very unusual environment. Tentative assignment of the signals as being due to the phosphoenzyme was their dependency on  $\text{Ca}^{2+}$ , their lack of stability and the observation that their combined intensity was less than expected from complete labelling of the enzymatic sites. In accord with kinetic evidence that all steps in the catalytic pathway are reversible, incorporation of inorganic phosphate into sarcoplasmic reticulum in the presence of a calcium gradient produced signals identical to those obtained from ATP in the absence of a gradient.

E. Ouabain sensitive ATPase activity in sarcolemma prepared from canine myocardium. The ouabain sensitivity of cardiac sarcolemmal (Na<sup>+</sup>K<sup>+</sup>)-dependent ATPase prepared from young and aged dogs was measured in conjunction with studies of the digitalis responsiveness of isolated muscle. Data obtained from 3 young and 3 aged dogs show no significant difference in the dose response curve of ATPase to ouabain. For both, the apparent inhibition constants  $\approx 3.5 \times 10^{-6}$  M. Consequently, differences in muscle responsiveness to ouabain are likely to reflect differences in the density of ouabain receptors rather than differences in binding affinity.

Significance to Bio-medical Research and to the Program of the Institute: Investigation of the catalytic pathways coupled to active cation transport should lead to a better understanding of how cells are able to establish and maintain the chemical gradients necessary for accomplishing physiologic work. This information should in turn be useful in distinguishing age-related alterations in the functional capacity of cellular membranes and promote a clearer understanding of the mechanisms responsible for those alterations.

Proposed Course of the Project: The rapid initial phase of  $\text{Ca}^{2+}$  accumulation in sarcoplasmic reticulum obtained from hearts of young adult and aged rats will be measured to determine if reduced steady state rates in aged preparations arise from a reduction in initial binding rates. Possible alterations in the efficiency of pumping will be investigated by measuring  $\text{Ca}^{2+}$  transport and ATPase activity in preparations which have been purified and reconstituted in an artificial phospholipid milieu. The effects of metal ions on the partial reactions of sarcoplasmic reticulum and electric organ ATPase will be further characterized by rapid kinetic methods.

Publications:

Froehlich, J. P., Sullivan, J. V., and Berger, R. L.: A chemical quenching apparatus for studying rapid reactions. Anal. Biochem. 73: 331-341, 1976.

Froehlich, J. P., and Taylor, E. W.: Transient state kinetic effects of calcium ion on sarcoplasmic reticulum adenosine triphosphatase. J. Biol. Chem. 251: 2307-2315, 1976.

Froehlich, J. P., Lakatta, E. G., Beard, E., Spurgeon, H. A., Weisfeldt, M. L., and Gerstenblith, G.: Studies of sarcoplasmic reticulum function and contraction duration in young adult and aged rat myocardium. J. Molec. Cell. Cardiol., in press.

NIA ANNUAL REPORT  
July 1, 1976 to September 30, 1977  
Gerontology Research Center  
Laboratory of Behavioral Sciences

Suppose that one is following a cohort of people throughout some period in their lives and observes that there is a systematic change that occurs within the group as it grows older. It is tempting to attribute that change to maturation. But is that necessarily so? Unfortunately, the answer is no. At least one other factor could account for the effect: The change could be due to a cultural process which is occurring throughout the measurement period. For example, if one sees that the incidence of a given disease increases with age in a group of subjects followed longitudinally, he will be tempted to call that a "disease of aging." But it is equally possible that some factor or factors is operating generally within the society to increase vulnerability and that the longitudinal cohort is merely reflecting a cultural trend. It is possible, with appropriate experimental designs, to tease out these effects and to separate longitudinal processes from cultural ones. In order to see how these mechanisms can be isolated, consider the following example: Three groups of subjects are studied. Group A is a longitudinal sample which is followed for 20 years from age 20 to age 40 during which it is observed that the disease incidence has risen monotonically. Group B is a cross-sectional sample ranging in age from 20-40 which is surveyed once at the time that the subjects in Group A are 20. Group C is another cross-sectional sample ranging in age from 20-40 years which is surveyed once when the subjects in Group A are 40. If the disease incidence is age-related, all three groups will show a monotonic age-related increase in incidence. If the disease is cultural, its incidence will be lower in group B than in group C, and its incidence will be similar among members of group B and among group A subjects who are now 40 years old. An analysis of certain personality traits among the men in the Baltimore Longitudinal Study group (age range 17 to 98 years) has found evidence for both of these processes over the 16 year period from 1958 to 1974. General activity and masculinity show maturational changes: General activity increased for men in their 20s, but declined steadily for men 50 years or older at the time of first measurement. Masculinity declined steadily for all age groups. Three traits, thoughtfulness, personal relations and friendliness showed declines which reflect cultural processes: Over the 16 year observation period, men of all ages become less tolerant of others and less interested in reflective, introspective thinking.

There is an enduring interest in this laboratory in age changes and differences in problem solving ability. Two studies in this area are especially noteworthy. A longitudinal study of 238 men who ranged in age from 26 to 84 years at the time of first measurement were retested on their abilities to solve conceptual problems seven years later. Men in the young age groups improved in their performance whereas older men showed a decline in reasoning. Furthermore, there were age-related declines in effectiveness of problem solving with the greatest declines occurring among the older men. These findings add to a growing body of data which show age-related declines in reasoning ability. Daydreaming often is conceptualized as one mechanism people use to solve problems. Previous research in this laboratory studied this behavior in a cross-sectional sample of men. A recently completed

study examined cross-sectional differences in a cohort of women ranging in age from 17 to 92 years. These results showed that women daydream more than men, and they daydream with greater intensity: This is true at all ages. Problem solving daydreams were the most common types at all ages for women whereas this was true only for men over the age of 30: Among younger men sexual daydreams were most common. Women showed a consistently lesser tendency to have sexual, heroic or bizarre daydreams than did men. Problem solving daydreams declined with age as it did for men, but sexual daydreams decreased with age more for women than for men. The similarity in age patterns between the sexes is impressive.

Several basic studies of age differences in psychophysiological performance have been completed during the past year. One study compared young and old men in terms of their electrodermal responses. The findings indicated that some of the age-related differences one sees such as the higher electrical resistance and lower negative skin potentials of older men may be due to age differences in skin hydration as well as relative magnitudes of sweat gland and epidermal potentials: In older subjects epidermal potentials may be higher than sweat gland potentials, and this is the reverse of what one finds in younger men. Studies of dopaminergic, age-related differences in the nigro-striatal system of the brains of rats indicate important differences which express themselves in behavioral differences as well. Old rats show less responsivity to chemically-induced stimuli which provoke turning behavior. Results of preliminary studies suggest that this effect may be related to a reduction in the neurotransmitter system rather than to the receptor system. Finally, in this series of studies we have completed an analysis of the effect of voluntary exercise on the life-span of the rat. The results show that exercising male animals had a 20% longer life-span than did less active control male animals (24.6 months vs. 20.7 months). For females the differences were 10% (29.2 months vs. 26.2 months). Clearly, the role of exercise in prolonging life should be explored further.

Experiments on the central neural mechanisms which mediate cardiovascular responses are an enduring interest of investigators in this laboratory. Studies with monkeys now in progress indicate that these animals can be trained to regulate their heart rates reliably. Electrophysiological studies of brain hippocampal function indicate that this area of the brain, which has been implicated in attentional and motor control, shows reliable changes during voluntary cardiac control. The prevalence of 4-8 Hz activity ("theta rhythm") tends to increase whenever an animal is successfully controlling its heart rate in either direction. These findings suggest that voluntary cardiac control in the monkey is not related to motor behavior, and the findings further suggest that hippocampal theta rhythm may reflect the presence of positive reward as well as motor action or attention.

Investigators in this laboratory have a major interest in the application of behavioral techniques in the rehabilitation of patients who suffer from chronic diseases. One study which was completed this year has shown that it is possible to facilitate recovery from a myocardial infarction among patients who are at high risk to injure themselves further by disregarding medical advice. These patients will cooperate fully in a rehabilitation program when they are given an opportunity to participate actively in

the design of the program; when they are allowed to set the goals of the program; and when they are given access to relevant clinical data on which rehabilitation decisions are made.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00061-15 LBS
PERIOD COVERED July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (80 characters or less) Behavioral Genetics and Aging		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <div style="display: flex; justify-content: space-between; margin-top: 100px;"> <div>             PI: Charles L. Goodrick              OTHERS: None           </div> <div>             Research Psychologist LBS GRC NIA           </div> </div>		
COOPERATING UNITS (if any) Baltimore City Hospitals		
LAB/BRANCH Gerontology Research Center - LBS		
St Learning and Problem Solving		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Md. 21224		
TOTAL MANYEARS: 1.10	PROFESSIONAL: .50	OTHER: .60
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div> <input type="checkbox"/> (a) HUMAN SUBJECTS         </div> <div> <input type="checkbox"/> (b) HUMAN TISSUES         </div> <div> <input checked="" type="checkbox"/> (c) NEITHER         </div> </div> <div style="margin-top: 10px;"> <input type="checkbox"/> (a1) MINORS    <input type="checkbox"/> (a2) INTERVIEWS       </div>		
SUMMARY OF WORK (200 words or less - underline keywords) <p>             The major purpose of this project is to determine <u>behavioral differences</u> throughout the lifespan for populations of <u>mice</u> which differ in genetic <u>structure</u>, and which also differ in <u>longevity</u>. The present topics of interest concern the relations of <u>growth rate</u>, maximal body weight and <u>longevity</u> for mice fed low levels of <u>dietary protein</u> and for groups fed adequate dietary protein, in addition to studies of <u>aging</u> and voluntary <u>wheel exercise</u>, and <u>ethanol</u> preference.           </p>		

Project Description:

Objectives: The principal objectives of this project are: (1) to determine group differences for behavioral traits and longevity among inbred strains of mice; (2) to determine heritability (degree of genetic determination vs. degree of environmental determination), mode of inheritance (e.g., overdominant, dominant, intermediate, or recessive), and number of segregating units (gene blocks) controlling a particular trait (e.g., longevity); and (3) to examine relative behavioral differences among mouse strains as aging progresses. Other objectives include determining the influence of diet (e.g., protein available) on behavioral traits, growth, and longevity; and identifying single-gene influences upon behavioral traits, growth, and longevity.

Methods Employed: Inbred mice (C57BL/6J and A/J) of a high degree of homozygosity are maintained under uniform environmental conditions. The animals are tested behaviorally during one period of their life span, viz, when mature, mature-old, or aged. Old age is determined as the 50% mortality point for groups maintained throughout their life span. Statistically reliable techniques have been developed to determine behaviors relevant to natural selection such as exploration, general activity level, emotionality, simple or complex problem solving ability and taste preference. The use of segregating  $F_2$  hybrid groups allows an estimate of the mode of inheritance, e.g., dominant or intermediate, and the number of gene blocks or segregating units controlling behavioral traits or life span. For studies in which protein intake is varied for groups of inbred and hybrid mice, isocaloric synthetic diets are used. Deprived animals receive 4% casein in their diets, whereas the control group receives a 26% casein diet. Numerous kinds of mutant mice are maintained on the C57BL/6J background at the Jackson Memorial Laboratories; Bar Harbor, Maine. Our work has concentrated on the albino, beige, yellow and obese mutations.

Major Findings:

A. A large portion of the world population has a maintenance diet which is characterized by very low levels of dietary protein. The effects of this diet are a reduction of body weight and a concomittant slowing of growth rate. We have been testing the hypothesis that the effect of protein malnourishment may increase longevity due to slowing of the rate of growth using inbred and hybrid mice as subjects. In addition, studies of behavioral differences as a function of dietary protein and stage of development are in progress. The longevity means as a function of genetic group and diet are given in Table 1. The main effect of group was highly statistically significant,  $F(2,281) = 16.31$ ,  $p < .01$ . C57BL/6J mice lived longer than A/J mice, and hybrid  $F_2$  mice lived longer than both inbred groups. In addition, mice fed low protein diets lived longer than mice fed normal protein diets,  $F(1,281) = 21.40$ ,  $p < .01$ . Because genetic group differences in longevity were greater for the low protein condition than the normal protein condition, the interaction of genetic group and diet was also significant,  $F(2,281) = 26.44$ ,  $p < .01$ .

The analysis of the growth rate measure (peak body weight divided by growth duration) obtained a highly significant main effect for diet,  $F$

(1,281) = 213.82,  $p < .01$ , (See Table 1). The growth rate was less for mice fed low dietary protein than for mice fed normal dietary protein.

For peak body weight, the main effect of genetic group was significant,  $F(2,281) = 296.73$ ,  $p < .01$ ; with C57BL/6J mice higher in peak body weight than A/J mice, and  $F_1$  hybrids higher in peak body weight than C57BL/6J mice. The main effect of diet was also highly statistically significant,  $F(1,281) = 1,316.63$ ,  $p < .01$ , with mice fed normal protein diets obtaining much higher peak body weights than mice fed low protein diets. The interaction of genetic group and diet was also significant,  $F(2,281) = 75.11$ ,  $p < .01$ , because peak body weight differences as a function of diet were greater for C57BL/6J mice and hybrid  $F_1$  mice than for A/J mice (see Table 1).

Growth duration varied significantly as a function of genetic group,  $F(2,288) = 17.19$ ,  $p < .01$  (see Table 1), with growth durations of C57BL/6J mice longer than those of A/J mice and hybrid  $F_1$  mice having longer growth durations than the two inbred groups. Groups fed low protein diets had significantly longer growth durations than groups fed normal protein diets,  $F(1,288) = 32.39$ ,  $p < .01$ .

Maximal longevity was greater for the low protein condition within each group than for the normal protein condition. Maximal longevity was greater for C57BL/6J mice than A/J mice, with the hybrid  $F_1$  group obtaining the greatest maximal longevity. The percentage of mice alive at 25 months, 27 months, and 29 months of age are given in Table 2, along with the absolute numbers. Combining over genetic groups, Chi square analyses (2 X 2 contingency tables) yielded highly statistically significant differences between mice fed low dietary protein and mice fed normal dietary protein. At each age significantly more of the survivors were from the low dietary protein condition than from the normal dietary protein condition. In addition, within all three groups, the variance of longevity was significantly greater for mice fed low dietary protein than for mice fed normal dietary protein.

The correlations among the variables of longevity, growth rate, peak body weight, growth duration, and last body weight are given in Table 3 for each combination of group and diet. All of the correlations of longevity and growth rate were negative, with 5 out of 6 attaining statistical significance. The slower the growth rate, the greater the longevity. The six correlations of longevity and peak body weight were all positive, but just two attained significance. However, 5 out of 6 correlations of longevity and last body weight were negative, with 2 attaining statistical significance. There was a tendency for greater longevity to be associated with higher peak body weight but with lower body weight at the end of the life span. All of the correlations of longevity and growth duration were positive, with 5 of the 6 attaining statistical significance. Mice which had long growth durations tended to be long lived. All correlations of growth rate and growth duration were significant and negative; the slower the rate of growth, the longer the growth duration. Peak body weight and



growth duration were positively related for all six correlations, with three statistically significant. In addition, all six correlations for peak body weight and last body weight were positive and statistically significant.

For the mean body weights at four stages in the life span, all main effects and interactions were highly statistically significant. The second order interaction genetic group X diet X age segment,  $F(6,836) = 6.80$ ,  $p < .01$  occurred because body weight increased differentially over the four age segments as a function of genetic group and diet. However, the mean percentages of body weight for these four age segments did not differ as a function of age or group. The mean percentages as a function of age segment were 23.2, 26.1, 27.5, and 23.2, reflecting an increment in body weight through age segment 3, then a decrement at the last body weight measure.

It was concluded that for mice, slowing the rate of growth and increasing growth duration results in a significant increase in the life span, and the life span increment is not negatively related to peak body weight.

Table 1

Means and Standard Errors for Longevity, Growth Rate, Peak Body Weight, Growth Duration, and Body Weight at the Last Month Prior to Death and Maximal Longevity as a Function of Group and Dietary Protein

Group	Dietary Protein	Mean Longevity (mo.)	Mean Growth Rate	Body Weight (gm.)	Mean Peak Weight (gm.)	Mean Growth Duration (mo.)	Mean Last Body Weight (gm.)	Maximal Longevity (mo.)
C57	Normal	21.2 <sup>±</sup> .46 <sup>xx</sup>	2.95 <sup>±</sup> .06 <sup>xx</sup>	45.3 <sup>±</sup> .68 <sup>xx</sup>	15.6 <sup>±</sup> .31	36.7 <sup>±</sup> .71 <sup>xx</sup>	30	
	Low	24.3 <sup>±</sup> .84	1.74 <sup>±</sup> .06	28.3 <sup>±</sup> .57	17.1 <sup>±</sup> .64	23.4 <sup>±</sup> .73	36	
A/J	Normal	21.4 <sup>±</sup> .61	2.58 <sup>±</sup> .15	32.0 <sup>±</sup> .54 <sup>xx</sup>	13.8 <sup>±</sup> .53 <sup>x</sup>	26.0 <sup>±</sup> .54 <sup>xx</sup>	29	
	Low	22.7 <sup>±</sup> .78	1.58 <sup>±</sup> .09	23.0 <sup>±</sup> .23	15.7 <sup>±</sup> .55	18.5 <sup>±</sup> .48	31	
F <sub>1</sub>	Normal	24.0 <sup>±</sup> .64 <sup>xx</sup>	3.23 <sup>±</sup> .06 <sup>xx</sup>	50.6 <sup>±</sup> .58 <sup>xx</sup>	15.9 <sup>±</sup> .29 <sup>xx</sup>	35.8 <sup>±</sup> .78 <sup>xx</sup>	33	
	Low	28.1 <sup>±</sup> 1.00	1.60 <sup>±</sup> .08	28.9 <sup>±</sup> .43	19.8 <sup>±</sup> .72	22.6 <sup>±</sup> .52	41	

Low Protein vs. Normal Protein

<sup>x</sup>  $p < .06$

<sup>xx</sup>  $p < .01$

Table 2

Percentage of Mice Which Lived to 25, 27, and 29 months of Age as a  
Function of Group and Dietary Protein

Group	Dietary Protein	25 mo.		27 mo.		29 mo.	
C57	Normal	16%	8/49	6%	3/49	4%	2/49
	Low	43%	18/42	36%	15/42	24%	10/42
A/J	Normal	27%	13/49	6%	3/49	4%	2/49
	Low	51%	25/49	27%	13/49	14%	7/49
F <sub>1</sub>	Normal	41%	20/49	31%	15/49	18%	9/49
	Low	80%	39/49	59%	29/49	55%	27/49

Normal protein vs. low protein, all groups

$$\chi^2 = 22.98$$

$$\chi^2 = 25.30$$

$$\chi^2 = 27.56$$

$$\text{df} = 1, p < .01$$

$$\text{df} = 1, p < .01$$

$$\text{df} = 1, p < .01$$

Table 3

Correlations Among the Variables of Longevity (L), Growth Rate (GR), Peak Body Weight (PBW), Growth Duration (GD), and Last Body Weight (LBW), for Three Groups, Each at Two Levels of Dietary Protein

Group	Dietary Protein	N	L X		L X		L X		GR X		PBW X		GD X	
			GR	PBW	GD	LBW	LBW	LBW	LBW	GD	LBW	LBW	LBW	GD
C57	Normal	49	-.41 <sup>xx</sup>	+ .16	+ .48 <sup>xx</sup>	-.28 <sup>x</sup>	+ .01	+ .47 <sup>xx</sup>	+ .37 <sup>x</sup>		+ .42 <sup>xx</sup>			
	Low	42	-.57 <sup>xx</sup>	+ .28	+ .63 <sup>xx</sup>	+ .02	-.08	+ .01	+ .63 <sup>xx</sup>		+ .30 <sup>x</sup>			
AJ	Normal	49	-.04	+ .41 <sup>xx</sup>	+ .17	-.20	-.32 <sup>x</sup>	+ .38 <sup>xx</sup>	+ .36 <sup>x</sup>		+ .37 <sup>x</sup>			
	Low	49	-.67 <sup>xx</sup>	+ .09	+ .65 <sup>xx</sup>	-.60 <sup>xx</sup>	+ .17	+ .23	+ .38 <sup>xx</sup>		-.14			
F <sub>1</sub>	Normal	49	-.36 <sup>x</sup>	+ .15	+ .36 <sup>x</sup>	-.14	-.08	+ .52 <sup>xx</sup>	+ .27 <sup>x</sup>		+ .25			
	Low	49	-.63 <sup>xx</sup>	+ .29 <sup>x</sup>	+ .67 <sup>xx</sup>	-.18	+ .06	+ .25	+ .53 <sup>xx</sup>		+ .03			

<sup>x</sup>  $p < .06$

<sup>xx</sup>  $p < .01$

Significance to Bio-Medical Research and Program for the Institute: The study of the genetics of behavior and longevity allows an assessment of: (1) the mode of inheritance (i.e., dominant, intermediate, etc.) for the factor studied (2) the relative importance of hereditary and environmental factors; and (3) the number of genes or gene blocks which control the factor studied. Lack of adequate dietary protein is a condition which affects a large proportion of the world populations. This project attempts to determine the effect of diet (such as different proportions of protein in the total diet) during particular stages of the life span upon behavior and longevity for animal populations which differ in genetic constitution. Studies of single gene mutant animals are of importance because they allow the assessment of the importance of a specific genetic locus for physiological or behavioral factors.

Proposed Course of Project: Further inbred strains and F<sub>1</sub> hybrid groups are being studied to determine the generality of mode of inheritance of behavioral factors. Cross-sectional and longitudinal studies of mouse behavior will continue with various mouse strains. The longevity of inbred and hybrid groups are also being determined. Experiments with low, normal, and high protein diets should determine: (1) the effect of varying protein diets upon behavior at maturity after access to these diets during various stages of development, and (2) the effects of a diet of low, normal or high protein at the time of measurement upon behavior.

Publications:

Goodrick, C. The mode of inheritance of emotionality in the mouse (Mus Musculus): Sex differences and the effects of illumination. Psychological Reports, 1976, 39: 247-256.

Goodrick, C. Behavioral differences in young and aged mice: Strain differences for activity measures, operant learning, sensory discrimination, and alcohol preference. Experimental Aging Research, 1975, 1: 191-207.

Goodrick, C. Behavioral genetics and aging. In Schneider, E. (Ed.): The Genetics of Aging, in press.

Goodrick, C. Body weight change over the life span and longevity for C57BL/6J mice and mutations which differ in maximal body weight. Gerontology, in press.

Goodrick, C. Body weight increment and length of life: II The effect of genetic constitution and dietary protein. Journal of Gerontology, in press.

Goodrick, C. Ethanol preference of inbred mice: Mode of inheritance and the effect of age on the genetic system. Journal of Studies on Alcohol, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01-AG 00062-04 LBS
PERIOD COVERED July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (80 characters or less) Daydreaming and Aging: Normative and Experimental		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  PI: Leonard M. Giambra Research Psychologist LBS GRC NIA Other: None		
COOPERATING UNITS (if any) Baltimore City Hospitals Morgan State University Towson State University College of Notre Dame of Maryland T., Traynor, Clinical Psychologist, Miami University, Oxford, OH		
LAB/BRANCH Gerontology Research Center - Laboratory of Behavioral Sciences		
SE Learning & Problem Solving		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Md. 21224		
TOTAL MANYEARS: .90	PROFESSIONAL: .70	OTHER: .20
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  The purpose of this work is to determine the parameters of <u>spontaneous-thought-intrusions (daydreaming)</u> and related mental activity such as <u>insight</u> , <u>intuition</u> , <u>mindwandering</u> , and <u>curiosity</u> . This is accomplished through the use of retrospective questionnaires and experimental manipulation. Topics of present interest are: (a) <u>sex differences</u> , (b) the relation of daydreaming to <u>depression</u> , (c) the relation of daydreaming to <u>personality</u> styles described in <u>temperament</u> terms, (d) <u>racial</u> , <u>religious</u> , and <u>socio-economic</u> influence on daydreaming, and (e) the relation of sexual daydreaming and sexual activity to the <u>menopause</u> .		

Project Description:

Objectives: The goals are: (1) to determine the incidence and content of daydreaming in specific subpopulations (e.g., young, middle aged, elderly) from various socio-economic classes, various races, etc; (2) to attempt to relate these differences in daydreaming to any underlying mechanisms such as physiological state, education, cultural values and beliefs, differential daily experiences, and (3) to investigate experimentally variables which normative studies have indicated may be potent determiners of daydreaming.

Methods Employed: The normative aspects of daydreaming are determined through the use of a structured self-report. Each participant completes a 21 item biographical questionnaire and a 344 item Imaginal Processes Inventory (IPI) which has both specific and general items concerning daydreams, nightdreams, fantasies, etc. To date approximately 1800 individuals from a wide variety of subpopulations have completed the IPI and bio-questionnaire. There are 28 scales in the IPI. Each item has five choices which are points on a continuum implying frequency or quantity. The choices were assigned values of 0, 1, 2, 3, or 4.

Major Findings:

I. Sex differences in daydreaming and related mental activity from the late teens to the early nineties.

Sex differences on the 28 scales of the IPI were of three types: (a) simple, as exhibited in the analysis of variance (ANOVA), (b) age related, as exhibited in the age-scale correlations, regression weights, and interactive effects as determined by the ANOVA's, and (c) inter-scale relational as exhibited by the interscale correlations and the factor patterns of the scales. If for each IPI scale one takes the mean of the age group means for each sex separately, then takes the female-male difference, then ranks those differences for all scales from largest positive (female larger) to largest negative (female smaller), one can obtain a summary of the "simple" effects of sex. The largest differences between the sexes occur on the two scales of curiosity with females showing more interpersonal curiosity and less impersonal or mechanical curiosity. The unexpected finding of the present study was that males showed equal strength of interpersonal and impersonal curiosity while females showed curiosity of an interpersonal nature far exceeding that of an impersonal or mechanical nature. When sex was made a marker variable, the male and female samples combined, and a factor analysis performed, a single "sex difference" factor resulted with interpersonal and impersonal curiosity occupying opposite poles of the factor. If one looks upon congenital curiosity as general or undifferentiated it would appear that the socialization process has acted to enhance the female's curiosity about people and reduced her curiosity about things while for males such socialization pressures may be either ineffectual or absent.

Other scales reflecting mental or attentional process on which females were significantly higher were distractibility and perceived rate of mentation; also, females were significantly lower on need for external stimulation. The Age by Sex interaction was significant for distractibility with males

showing more distractibility than females in the 35-44 year age span. Age-scale regression analyses indicated that females had a somewhat slower drop in mindwandering, boredom, and distractibility with increasing age and a somewhat faster decrease with increased age in need for external stimulation. The basis, empirical or theoretical, for these sex differences on attentional processes is a puzzle.

Both frequency of daydreaming and absorption in daydreaming find higher levels in females at essentially every period of adulthood investigated. For females both frequency of and absorption in daydreaming had weaker negative correlations with age; this was also evident in smaller age-scale linear regression weights for females.

Four daydreaming content areas showed simple sex differences. Females showed a lesser likelihood of daydreams of a sexual, heroic, or bizarre-improbable nature and a greater likelihood of daydreams of a problem-solving nature. The Bizarre-Improbable Daydream scale was the only content scale which loaded on the single "sex difference" factor of the factor analysis. Only sexual daydreaming content showed a significant Age X Sex interaction in the ANOVA; the difference between males and females increased with age. For sexual daydreams the linear age-scale regression weight was significantly more negative for females than for males. For females sexual daydreaming levels were essentially unchanged within the 17-34, 35-49, and 50-59 year old intervals with relatively large drops between these intervals. The 45-49 year span coincides with the advent of menopause while the 50-54 year span coincides with the termination of menopause. Could this drop in sexual daydreaming be directly related to this important physiological change state of women? For problem solving daydreams the linear age-scale regression weight for the males was significantly more negative than for the females which was not different from zero. With the exception of hostile content, the daydream content scales showed no sex differences for the age-scale correlation when controlled for frequency of daydreaming. The linear age-scale regression weight for males was significantly greater, negatively, for hostile daydream content. This was due essentially to 17-23 year old males having a substantially greater likelihood of hostile daydreams than any other age-sex group. The fear of failure, guilt, and achievement-oriented daydream contents showed no significant simple sex effect although the achievement-oriented content sex difference was between the .05 and .10 level with males showing the higher values. Achievement-oriented daydreams also had a higher correlation with sex than problem-solving content which did have a significant simple sex effect.

When daydreaming contents are ranked within each sex for each half decade of life there were sex differences in the ages when contents change rank. Thus problem solving content shifted to a tie for first rank at age 24 for males whereas for females problem solving content always was first rank. Sexual daydreams for males were at first rank for 17-23 year olds and tied for first rank for 24-29 year olds and at second rank beginning at age 30; however female sexual daydreams were never higher than second rank. After age 30 for females the relative importance of sexual daydream content dropped sooner and more often (ages 50, 60, and 70) than for males (ages 65 and 75).



Unexpected, achievement oriented content was of the same relative rank from ages 17-39 for both sexes. Also, unexpectedly, at age 40, males and females diverged with achievement rising in rank for females but not for males. In summary, it is clear that females reported more daydreams involving problem solving and this content type achieved its highly prominent status earlier in the female's lifetime. Females reported less daydreams of a sexual nature and this type of daydream faded more quickly in importance for females than males; furthermore even in the youngest female age groups sexual daydreams did not achieve highest prominence as they did with males. Females reported a lesser likelihood of heroic, achievement-oriented, and bizarre daydream content; yet later in life achievement oriented daydreams was relatively more prominent in the female than in the male repertoire. No sex differences existed in contents of a guilt, fear of failure, and hostile nature although 17-23 males had substantially more daydreams of a hostile-aggressive nature than any other group, male or female, or any age.

## II. Independent dimensions of depression: A factor analysis of three self-report depression measures

Covariance studies of objective depression measures have concentrated on total scores. This approach is relatively insensitive in specifying whether these instruments measure the same sub-aspects of depression. To investigate this question a factor analysis was performed of the items of the Beck Depression Inventory and the Zung Self-Rating Depression Scale and lists A, B, C, and D of the Lubin Depression Adjective Check Lists. Subjects were 91 college students and 29 correctional institution inmates. Following a Varimax rotation, four clearly interpretable multimeasure factors resulted. The most salient factor was labeled "Depression: Affective Malaise." Earlier studies have also shown this to be a dominant and reliable dimension of depression. The other factors were: "Suicidal Ambivalence," "Appetite-Weight Loss," and "Fatigability." Females showed greater "Fatigability" associated with depression. Factors specific to the Beck and Zung measures were also found suggesting that the different emphases of these instruments, intensity/severity vs. frequency of symptoms, may contribute very specific depression indicators. This indicates perhaps, that both intensity and frequency of symptoms ought to be considered to obtain a "best" objective measure of depression.

## III. Depression and Daydreaming: An Analysis Based on Self-Ratings

The purpose of this study was to investigate the relationship between depression and daydreaming characteristics in a non-hospitalized sample. Level of depression was determined by the Beck Depression Inventory, The Zung Self-Rating Depression Scale and the Lubin Depression Adjective Check Lists. Daydreaming characteristics were determined from a self-report retrospective questionnaire, The Imaginal Processes Inventory. The sample consisted of 91 university undergraduates and 29 male correctional institution inmates. Analyses involved both full-scale depression scores and the items of the depression measures. The full-scale global measures of depression were found to be directly related to the neurotic, anxious, dysphoric, and negative dimension of daydreaming. A search for specific relationships between components of depression and factors of daydreaming yielded six such

specific relationships. Specific direct relationships were noted between mental agitation and distractibility, between indecisiveness-personal devaluation and mental slowing, and between personal devaluation-poor body image and fear of failure daydreams. Inverse relationships were noted between a sense of punishment and useful-positive aspects of daydreaming and between psychomotor-activity level and internally stimulated mental activity. The suicidal ambivalence, appetite-weight loss, and fatigability dimensions of depression were found to be unrelated to daydreaming.

IV. A factor analytic study of daydreaming, imaginal process, and temperament: A replication on an adult male life-span sample. The relationship of an individual's temperament to his pattern of daydreaming and related mental activity has been investigated in two separate studies by Singer and Antrobus (1963, 1972). These studies have demonstrated, in college students, that temperament traits such as thoughtfulness, objectivity, masculinity, and emotional stability are related to daydreaming and related imaginal/attentional activities. It has been demonstrated in this laboratory that daydreaming and related mental activity are age dependent. This study determined the generality of the Singer and Antrobus findings for males aged 24 to 91 years.

One hundred and seventy males aged 24-91 years were measured on daydreaming and related mental activity using the Imaginal Processes Inventory and on temperament using the Guilford-Zimmerman Temperament Survey. A factor analytic approach was used. Five daydreaming-temperament factors of Singer and Antrobus were replicated. Age loaded on only one of these factors, "Neurotic-Anxious Absorption in Daydreaming." The age relation was negative; neurotic-anxious absorption in daydreaming decreased with increased age. The four non-age factors which were replicated for a total adult life-span were "Controlled-Thoughtfulness," "Masculinity-Femininity," "Thinking Introversion," and "Social Extroversion."

V. Religious, racial, and demographic differences in daydreaming Data from more than 1800 individuals who have responded to the Imaginal Processes Inventory are currently being analyzed to determine unconfounded differences attributable to religious, racial, and socio-economic backgrounds.

VI. Interrelationships among daydreaming characteristics, health, estrogen usage in pre-menopausal, menopausal, and post-menopausal women. The study on sex differences (see I above) found in women a "quantum" drop in sexual daydreaming at age 50 as compared with the previous 15 years. Since this is the menopausal transition period for most women it was decided to investigate this potential relationship more closely. Information on daydreaming, menopausal state, and estrogen usage is currently being collected on a sample of 250 women 40 to 60 years of age.

Significance to Bio-Medical Research and Program of the Institute: The study of daydreaming is fundamentally a study of thought processes. In order to understand fully the thought processes of man, the total spectrum

of those processes needs to be examined. In addition, it is important to know how this wide spectrum is affected by aging. Thus the study of daydreaming in adults, along with other variables, such as differences in age, socio-economic status, attitudes, etc., may help us understand the fundamental processes which underlie all these behaviors.

Proposed course of project

1. Intra-individual changes as a function of age will be studied.
2. Experimental studies of age differences in daydreaming, mind-wandering, distractibility, and intuition will be initiated.

Publications:

Giambra, L. M. Daydreaming about the past: The time setting of spontaneous thought intrusions. The Gerontologist, 1977, 17: 35-38.

Giambra, L. M. & Martin, C. E. Sexual daydreams and quantitative aspects of sexual activity: Some relations for males across adulthood. Archives of Sexual Behavior, in press.

Giambra, L. M. Adult male daydreaming across the life span: a replication and further analyses. International Journal of Aging and Human Development, in press.

Giambra, L. M. Sex differences in daydreaming and related mental activity from the late teens to the early nineties. International Journal of Aging and Human Development, in press.

Giambra, L. M. Independent dimensions of depression: A factor analysis of three self-report depression measures. Journal of Clinical Psychology, in press.

Giambra, L. M., & Traynor, T. D. Depression and daydreaming: An analysis based on self-ratings. Journal of Clinical Psychology, in press.

Giambra, L. M. A factor analytic study of daydreaming, imaginal process, and temperament: A replication on an adult male life-span sample. Journal of Gerontology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF</b> INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00063-10 LBS												
PERIOD COVERED July 1, 1976 to September 30, 1977														
TITLE OF PROJECT (80 characters or less)  Learned Modification of Visceral Function in Animals														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">J. A. Joseph</td> <td style="width: 30%;">Staff Fellow</td> <td style="width: 30%;">LBS, GRC, NIA</td> </tr> <tr> <td>Other:</td> <td>B. T. Engel</td> <td>Chief, Lab. of Behavioral Sciences</td> <td>GRC, NIA</td> </tr> <tr> <td></td> <td>G. S. Roth</td> <td>Research Chemist, Endocrinology</td> <td>CPB, GRC, NIA</td> </tr> </table>			PI:	J. A. Joseph	Staff Fellow	LBS, GRC, NIA	Other:	B. T. Engel	Chief, Lab. of Behavioral Sciences	GRC, NIA		G. S. Roth	Research Chemist, Endocrinology	CPB, GRC, NIA
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COOPERATING UNITS (if any) D. A. Kristt, Johns Hopkins School of Medicine														
LAB/BRANCH Laboratory of Behavioral Sciences														
SITE Psychophysiology														
INSTITUTE AND LOCATION GRC, NIA, NIH, Baltimore, Maryland 21224														
TOTAL MANYEARS: 4.60	PROFESSIONAL: 1.10	OTHER: 3.50												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords)  The purpose of this project is to investigate the role of the <u>central nervous system in behavior</u> . In some experiments we examine the contribution of <u>somatic factors in instrumental cardiovascular conditioning</u> : <u>Brain</u> areas of special interest are the <u>hippocampus</u> , the <u>caudate nucleus</u> and the <u>pyramidal tract</u> ; subjects are <u>monkeys (Macaca mulatta)</u> . In other experiments we examine the age-related changes in <u>pre- and post synaptic</u> portions of the <u>nigrostriatal pathways</u> using <u>behavioral</u> and <u>biochemical</u> analytical methods. The present topics of interest include possible alterations (a) in development of <u>post synaptic denervation hypersensitivity</u> , (b) the specific of <u>dopamine receptor binding</u> , (c) <u>adenyl cyclase</u> production and (d) <u>synaptic</u> release mechanisms that might occur with age.														

## Objectives

A. To determine the neural mechanisms involved in cardiovascular conditioning in monkeys.

B. To analyze the age differences in biochemical and structural characteristics of the brain in relation to behavior.

## Methods Employed

A. In numerous experiments, in a variety of lower animals, the hippocampus, a neocortical structure, has been implicated in somatic control. This structure has a dominant frequency of 4-8 Hz which can be recorded from the pyramidal cell layer of the dorsal hippocampus when the animal performs or intends to perform a voluntary movement. This frequency has been designated as "theta" activity or rhythmic slow activity.

As a first attempt at deducing some of the possible CNS mechanisms that might be involved in the instrumental control of heart rate, electrodes were chronically, bilaterally implanted in the hippocampi of monkeys operantly conditioned to speed and to slow their heart rates. Since the hippocampus seems to be related to movement, and since heart rate changes might be related to changes in movement, more theta activity should be seen during heart rate speeding sessions than during slowing sessions.

Each session comprised by a 512 second baseline period and a 2048 second testing period. Three types of sessions were examined: "speeding sessions" during which the animal was operantly trained to speed its heart, "slowing sessions" during which the animal was trained to slow its heart, and no feedback sessions in which the animal was not required to speed or to slow its heart. It simply sat in the closed booth throughout the 512 sec baseline period and the 2048 sec training period. Information on speeding and slowing was provided by lights. A red light indicated that the animal was to slow its heart and a green light indicated the speeding condition. A white light functioned as a reinforcement light to indicate to the animal that it was performing correctly. A 10 ma electric shock was delivered to the tail once/8 sec for incorrect responding. Speeding and slowing sessions were further subdivided into "push" and "non push" sessions. Push sessions were designated as those in which the animal was required to change its heart rate until it was at some specified level below (slow) or above (speed) its baseline heart rate (10, 15, 20%). During non-push sessions this was not done; the animal only had to keep its heart rate below or above baseline.

For purposes of analysis the sessions were divided into 16 blocks of 128 second duration and the baseline was divided into 4 blocks of 128 sec duration. Heart rate, systolic, and diastolic blood pressure were recorded as well as samples of hippocampal EEG for the first 10 sec of each block. The "raw" hippocampal activity was filtered by a band pass filter at the 4 to 8 Hz range and then integrated. The activity in this range was defined as theta and the amount of theta for each 128 second block was computed.

Additionally a fast Fourier analysis was carried out on the 10 sec samples of hippocampal activity so that the energy distribution from 1-50 Hz could be examined. Gross motor activity was scored visually by a trained observer during the first 10 sec. of each block.

B. It has been reported previously that a surgically induced lesion in the substantia nigra of young animals results in a substantial decline in the level of a dopamine (DA) in the caudate nucleus of the lesioned side. The caudate nucleus has been shown to be very important in the control of movement, and an imbalance in the neurotransmitters between the lesioned and unlesioned nigrostriatal pathways produces a directional dominance in the animals' movements such that after injection of certain drugs such as apomorphine (a DA receptor agonist) or amphetamine (which promotes the release of DA) animals can be induced to turn circles. These rotations can be accurately measured with a device called a rotometer. Numerous studies have shown that: (a) amount of depletion; (b) DA receptor activity; (c) development of denervation receptor hypersensitivity; and (d) presynaptic release events can be gauged by the strength and directionality of the rotational behavior. This behavior seems to be almost exclusively modulated by the nigrostriatal system and by dopaminergic-cholinergic interactions within this system.

In the behavioral experiments, lesions are produced unilaterally (left side) in the substantia nigra of young (4-6 mo) and old (25-29 mo) males and females following several prelesion baseline measures of rotational behavior. Following a 7-10 day recovery period baseline rotations are again examined to determine if there are any effects of surgery. After this the animals are given a graded series of doses of: (A) amphetamine (0, .5, 1, 2, 5 mg/kg); (B) apomorphine (0.25, 2, 5 mg/kg); and (C) apomorphine (5mg/kg) followed by haloperidol (a dopamine receptor antagonist) (.25, .5, 1, 2 mg/kg). Each animal receives all doses of all drugs (2 doses at each level), but he receives the complete series of one drug before being given the next.

In other experiments young and old animals are given amphetamine prior to lesioning to determine if a natural imbalance in dopamine may be found between the left and right caudate nuclei and if this imbalance is enhanced or diminished with age.

In a third set of experiments changes in the numbers of dopamine receptors in the caudate nuclei are being assessed through binding studies carried out with tritiated haloperidol and some assessment also is being made of adenylyl cyclase and phosphodiesterase in the caudate nuclei young and old rats.

Finally in a fourth group of experiments the rotational behavioral method is being used to examine what pharmacological agents might be utilized to overcome any defects that are present in old animals. As an example, it is known that if haloperidol is given to lesioned animals for 7 days and then withdrawn, turning behavior can be elicited with a lower dose of apomorphine or amphetamine. This is presently being examined in young and old rats.

Major Findings

A. Analysis of the baseline data from two monkeys showed that baseline heart, theta, and activity rates did not differ (i.e., heart rates from the baselines of each session were approximately the same) prior to each type of session as shown in Table 1 for each animal

	M-6			M-7		
	NF	Fast	Slow	NF	Fast	Slow
Heart rate	183.9	180.6	180.9	168.5	167.0	168.5
Theta	116.5	115.4	111.5	138.2	136.5	136.7
Activity	7.7	7.0	7.7	4.5	4.0	4.5

The data for the push and non push sessions have been considered together for purposes of the initial analysis. Table 2 shows the summary of correlations between the various dependent measures under the slow, fast, and nofeedback conditions for each animal. All correlations are based on changes from baseline.

(Symbols: H = heart rate;  $\Theta$  = hippocampal theta; A = activity).

	M-6			M-7		
	H $\Theta$	HA	$\Theta$ A	H $\Theta$	HA	$\Theta$ A
S	-.37*	.23*	.01	-.09	.34*	-.11
F	.23*	.33*	.11	.48*	.34*	.14
NF	-.54*	.23*	-.14	.43*	.34*	.24*

Correlations marked with an asterisk are significantly different from zero ( $p < .01$ ). Theta is inversely related to HR changes during slowing, that is, the greater the heart rate deceleration the more theta increased (-.37; -.09 respectively). On the other hand, increases in theta were related to increases in heart rate during the speeding sessions (.23; .48 respectively). Under nofeedback the correlations were positive for M-7 and negative for M-6 even though both animals showed a drop in heart rate (-3.8 beats/min and -12.0 beat/min respectively). Correlations between heart rate and activity tended to be fairly constant and positively related among the three conditions for both monkeys. Relationships between theta and activity remained low and statistically nonsignificant under all conditions. These data suggest that: (a) theta is virtually unrelated to motor activity as it is measured in this experiment; (b) motor activity changes alone cannot account for the differences in heart rate seen under the different conditions. Thus, somatic activity, *per se*, is not a sufficient condition for performance of the task, and using motor activity measures alone, one cannot distinguish between slow and fast conditions. It would appear that once a steady state of activity is reached during a session, it then becomes a relatively unimportant variable in mediating the ensuing cardiovascular changes; (c) The relationship between theta and heart rate does change under the different conditions. It is negative during slowing for both subjects and positive during speeding. Theta seems to be more complexly related to HR than we had originally believed: It is not simply another index of motor activity. Instead it (theta activity) seems to be related to how well the animal is performing during conditioning. Animal 6, which slowed more reliably than it

sped, showed a stronger relationship during the former condition than the latter, while for animal 7, which sped better than it slowed, the correlation is reversed. Thus, theta activity may function to gate or process other variables important for the mediation or control of this learned response. Two examples of such controlling variables are attention and motivation.

B. All of the major findings have come from the behavioral examinations. The biochemical studies have just begun.

1. Since all animals (32; 8 old males, 8 young males, 9 old females, 7 young females) were lesioned in the left substantia nigra, DA levels in the left caudate nucleus should be diminished. When amphetamine is given, DA release is promoted on the unlesioned side and the animal turns toward the left. It has been shown in young animals that the greater the strength of turning toward the lesioned side the greater the imbalance in DA that exists between the lesioned and unlesioned striatum. Results of the analysis of baseline turning rates showed that old animals emitted less overall turning than did young animals ( $\bar{X}$  = 2.0 turns/30 min (T) old;  $\bar{X}$  = 16.5 T, young), and that the number of left and right turning rates were equal (old  $\bar{X}$  = 2 T left,  $\bar{X}$  = 2 T right; young,  $\bar{X}$  = 13 T left,  $\bar{X}$  = 20 T right). Under the influence of amphetamine the animals began to turn increasingly toward the left as a function of dose. This occurred in both old and young animals. A direct comparison of the magnitude of the differences in left turn behavior with increasing doses of amphetamine was difficult to make because there were age differences in initial turning behavior as well as in drug induced turning behavior. The data was therefore transformed and a strength of response measure was computed by taking the left turns for each animal and dividing them by the right turns. Baselines were equated (old .88, young .85) and the resulting analysis of variance showed that old animals showed lower ratios of left/right turns and this was true throughout the dose range ( $\bar{X}$  dose ratio overall: Old = 5.5, young = 24.0). There were no differences between males and females in response strength. Old animals tended to increase both right and left turns as the dose of amphetamine was increased, while young animals selectively increased left turns. These findings suggest that there is a defect in DA responsivity at either the pre or post synaptic receptor on the unlesioned side, since histological analysis of the brain of these animals showed that the lesions were all of the same size. It is difficult to say where the deficit lies but on the basis of the apomorphine data discussed below it is likely that the deficit may be in the releasing mechanism.

1. Since apomorphine is a DA receptor agonist, it should affect both caudate nuclei if injected intraperitoneally. Since the caudate on the lesioned side develops degeneration hypersensitivity, apomorphine should affect it more strongly and the animal should exhibit contralateral turning (i.e., away from the lesion side) behavior. A deficit in: (a) development of supersensitivity, or (b) receptor number or both should be reflected in diminished responding. An analysis of variance computed on the ratios of the contralateral to ipsilateral turns showed that there was no age effect



( $F < 1.0$ ) even though the drug increased turning behavior ( $F > 4.0$ ,  $p < .0001$ ). These findings suggest that the deficit seen in the old animals shown earlier probably is mediated presynaptically. Additionally, it could mean that there are natural deficits in release of dopamine in old animals in which case the receptors may be in a constant state of hypersensitivity so that any decrease in these numbers might not be seen with direct agonist stimulation.

#### Significance to Bio-medical Research and the Program at the Institute

A. The present research, which is attempting to delineate the neural mechanisms involved in the behavioral control of the cardiovascular system is very important for specifying the constraints of this system to such control. This information should enable us to more effectively understand behavioral control procedures which we also are studying clinically in man.

B. One consistent finding that has been reported previously is that central catecholamine neurons decrease with age. Numerous experiments have demonstrated: (a) a reduction in the content of dopamine in the caudate nucleus of senescent mice; and (b) a profound reduction in tyrosine hydroxylase in aged animals. It also has been seen that the main symptoms of Parkinsonism (a disease of the aged) is the result of degenerative changes in the zona compacta of the substantia nigra. Thus, investigation of alterations in striatal dopamine and its interaction with other neurotransmitters as a function of age is an extremely important topic for research. A rotational model, because of its simplicity and dependency upon one clearly delineated neural substrate, is a very important tool for investigating these changes. Its importance is that, for the first time we can begin to tease out some relationships between CNS and behavioral events that are relatively free from acquisitional or motivational effects. More importantly, by using the model with various pharmacological agents, we can begin to see how some of these defects might be altered or ameliorated.

#### Proposed Course of the Project

A. The present experiment will be continued so that the contributions of the hippocampus during instrumental heart rate conditioning can be assessed more definitively. Other CNS areas concerned with somatic control will be examined during cardiovascular conditioning in order to determine their involvement in mediating this response. We plan to look at the caudate nucleus and the pyramidal tracts next. If the analysis of the present experiment reveals that the hippocampus does play a role in influencing performance during cardiac conditioning, then experiments of two types will examine this more extensively. Recordings will be made from the hippocampus during acquisition of the heart rate response in order to determine if hippocampal response frequencies during acquisition differ from those seen after stable performance has been achieved. Theta production will be influenced by electrical stimulation of the medial septal nucleus and instrumental conditioning in order to determine how increases or decreases in theta production might influence performance during conditioning sessions.

B. So far, the project has concentrated on the dopamine system. Further investigations will examine turning behavior after modifications on serotonergic, noradrenergic, or cholinergic systems. In addition, control studies will be carried out to determine other factors that might account for the differences in turning behavior with age (e.g., differences in the rate of metabolizing the test drugs, etc.).

#### Publications

Engel, B. T., Gottlieb, S. H. and Hayhurst, V. F.: Tonic and phasic relationships between heart rate and somato-motor activity in monkeys. Psychophysiology. 13(4): 288-295, 1976.

Thorne, P. R., Engel, B. T. and Holmblad, J. B.: An analysis of the error inherent in estimating heart rate from cardiachometer records. Psychophysiology. 13(3): 269-272, 1976.

Engel, B. T.: Operant conditioning of visceral responses. In Proceedings of the VII Annual Conference on Behavioral Modification, Mexico City, Mexico, in press.

Joseph, J. A. and Appel, J. B.: Behavioral sensitivity to LSD: Dependency on the pattern of serotonin depletion. Pharmacology Biochemistry and Behavior. in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00064-16 LBS								
PERIOD COVERED LBS                      July 1, 1976 to September 30, 1977										
TITLE OF PROJECT (80 characters or less)  Problem Solving and Aging										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT										
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%; vertical-align: top;">Pis:</td> <td style="width: 35%; vertical-align: top;">David Arenberg,</td> <td style="width: 35%; vertical-align: top;">Chief, Learning &amp; Problem Solving Section</td> <td style="width: 15%; vertical-align: top;">LBS GRC NIA</td> </tr> <tr> <td></td> <td style="vertical-align: top;">Leonard M. Giambra</td> <td style="vertical-align: top;">Research Psychologist</td> <td style="vertical-align: top;">LBS GRC NIA</td> </tr> </table>			Pis:	David Arenberg,	Chief, Learning & Problem Solving Section	LBS GRC NIA		Leonard M. Giambra	Research Psychologist	LBS GRC NIA
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COOPERATING UNITS (if any)  Baltimore City Hospitals										
LAB/BRANCH  Gerontology Research Center - LBS										
Sec  Learning and Problem Solving Section										
INSTITUTE AND LOCATION  NIA, NIH, Baltimore, Md. 21224										
TOTAL MANYEARS: 1.52	PROFESSIONAL: .50	OTHER: 1.02								
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords)  The primary purposes of this project on <u>aging</u> are to; (1) identify <u>reasoning processes</u> ; (2) determine how these processes <u>change</u> with age; and (3) develop techniques for reducing <u>age deficits</u> in reasoning performance. Reasoning is studied using <u>problem solving</u> procedures including <u>concept learning</u> and <u>concept identification</u> .										

Project Description:

Objectives: The general goals are to explore and identify reasoning processes in man, to determine in what ways these processes change with age, and to develop techniques for reducing age deficits in reasoning performance. In this project, reasoning is studied by using problem-solving procedures in which on-going solution behavior can be observed and quantified. Experiments are designed to answer such questions as: (1) Is effectiveness in acquiring relevant information affected by aging? (2) Is effectiveness in synthesizing available information affected by aging? (3) What kinds of solution strategies are used and in what ways are they related to age? (4) How does imposing a memory load affect solution strategies for young and old adults?

Methods Employed: Experiment V is a longitudinal study of concept identification. In this study, subjects select instances (examples) sequentially and are informed whether each selection is a positive example of the concept or a negative example. On the basis of the information elicited by these selections and their classification, the specific attributes of the concept can be identified. This procedure provides a measure of information gain for each selection. Ordinarily when subjects select instances, logically equivalent selections do not always result in equivalent information gains, and fortuitous selections can alter drastically the difficulty of a problem. A technique was developed which provides equivalent information gains for logically equivalent selections and avoids fortuitous gains in information. This technique also has two other important characteristics: (1) different types of problems which are logically equivalent can be constructed and compared, e.g., conjunctive concepts in which two attributes must both be present can be matched with two-attribute disjunctive concepts in which either attribute can be present; and (2) initial information gain can be manipulated because the experimenter can minimize or maximize the information gain for each selection. Experiment V was designed to determine age differences and age changes for six different types of problems determined by the number of attributes in the concept (one or two), and for two-attribute problems, the concept rule (conjunctive and disjunctive) and the amount of initial information gain.

Experiment XI is a concept study in which each subject solves a large number of problems. The literature in concept identification is based almost entirely on mean effects of subsamples in which each person solves one or a few problems. It has not been established that the variables which affect the mean performance of groups would affect an individual in the same way. When a person solves one or a few problems, his approach to reasoning can be characterized as unstable, variable, transient, and highly subject to chance occurrences. Solving many problems is expected to result in a steady state of performance. In that steady state, strategies can be more readily elicited and the effects of various independent variables on changes in strategy and on other performance measures can be studied. After solving 48 complete learning problems, and again after 96, 8 more problems are solved in which subjects "think out loud" throughout each problem. When individuals "think out loud" they are verbalizing all the thoughts about the

concept problem that are in their conscious awareness. They are specifically asked: (a) to relate the basis of their concept classification of the stimulus instance before them; (b) to tell if they had seen the stimulus instance earlier in the problem and if they remember its concept classification; (c) to relate how they use the feedback information about the correct classification of the stimulus instance before them to help them learn the concept; and (d) for any mnemonics they use to remember any and all aspects of a problem during the solution. The protocols of these "thinking-out-loud" problems constitute the primary data of this study. They are used to construct individual models of how a subject solves complex concept problems with which he is highly practiced. These models have as a general goal the prediction of each individual's performance on concept learning problems by specifying at each point in a problem how the individual will classify a stimulus instance as an exemplar or a nonexemplar of the concept. This general goal is met by succeeding at the more fundamental goals of specifying: (a) what information and past stimulus instances are stored in memory as well as for how long and what the retrieval cues are; and (b) how information on the exemplar status of a stimulus instance is utilized, inductively or deductively, to arrive at a solution to the problem, i.e., to identify the unknown concept. To achieve goals (a) and (b) a detailed specification of mechanisms is needed. These finely detailed mechanisms, appropriately connected in a flow diagram, constitute the individual's model. Comparisons of these mechanisms and their connections provide the means of specifying individual differences as well as age (generational) differences.

Major Findings: Experiment V is the longitudinal study of concept problem solving and age. Earlier, cross-sectional analyses had shown age differences in this task in each of the six types of problems which are administered. Not only proportions of problems correctly solved decreased over the 30s to 70s age range, but measures of effectiveness for problems solved correctly also decreased with age.

As of June, 1977, 238 participants could be included in preliminary longitudinal analyses of two sets of measures obtained approximately seven years apart. For this sample also, cross-sectional age differences showed a clear decline for each type of problem in both proportions correctly solved and in mean effectiveness of correct solutions.

Longitudinally, for the sample as a whole, mean total number of problems solved did not change. However, age was related to change; the young age groups improved somewhat, whereas the older groups declined.

The measure of effectiveness was calculated only for correct solutions. In order to examine change in this measure for each problem, only subjects with correct solutions both times could be included. As a result, these data represent a select subsample which is particularly biased (positively) for the older groups. That is, the old subjects who solved a specific problem both the first and second times were a highly select and unrepresentative subsample of their age peers in the study. Despite this bias, change (measured as a residual, i.e., that part of the second measure independent of the first measure) was related to age in all six types of problems. In

each problem type, larger declines were associated with higher ages. It should be noted that these correlations were low, and performance of many of the older problem solvers did not decline. But the consistency of the relationship between change and age over problems is substantial evidence that effectiveness in solving concept problems, even for the select subsample of men who solved a specific problem correctly both times, declines with age. Essentially the same results emerged when only those participants with a college degree were included in the analyses.

In all of these problems, subjects select the instances. The effectiveness measure is based upon the amount of information each selection could provide. Detailed analyses of these selections are planned and should identify the sources of difficulty which contribute to the declines in effectiveness.

In Experiment XI, complete data for the basic 96 problems and 16 "think aloud" problems have been collected on (a) men aged 12, 17, 20, 26, 40, 63, 71, 87, and 97 years; and (b) women aged 13, 18, 26, 35, 51, 66, 81, 89, and 90 years. Data are currently being collected on a man 106 years old. Yet to be collected are data on men in the 30's and 50's and women in the 40's and 70's.

A model has been completely specified for the 96 year old man. This predictive model shows a severely limited memory component. This memory limitation is dealt with by beginning each concept problem with a preset hypothesis which takes one of two alternate, but logically identical, forms. The requirement to guess the exemplar status of a stimulus instance is met by using this preset hypothesis. This hypothesis results in errors in identifying the exemplar status of a stimulus instance, and these errors lead to modifications of the hypothesis. The form of a modification is an exception to the preset hypothesis. An exception is arrived at by comparisons to the preset hypothesis. An exception is arrived at by comparisons of the present instance with the previous instance and with the initial instance. After seeing ten instances the initial instance is forgotten. The first exception deduced is retained in memory for the duration of the problem. Other exceptions are retained for three more trials and then forgotten unless a reconfirmation of the exception occurs. If a reconfirmation occurs, the exception stays in memory for the remainder of the problem. If within three trials an exception is contradicted, then the exception is modified to a more specific form and retained for three more trials. This approach has the benefit of requiring a minimal memory for new information and of reducing the errors made in guessing the exemplar status of new stimulus instances. The approach, however, fails in that it seldom leads to a correct identification of the concept.

To determine if this 96 year old man's failure to finally solve the concept problems was the sole result of his memory limitation, and not also of a deficit in inductive or deductive reasoning, he was given the same set of problems again with a reduced memory load. On these problems he was told at the beginning of each problem the two attributes of the stimulus instances which were relevant to solution. This modification greatly reduced his

memory load and left problem solution essentially to making of correct inferences. In this reduced memory condition he was able to solve the concept learning problems. Similar to the original problems, he began each problem with a preset hypothesis. In the modified problems, however, his hypothesis was based on a fundamental understanding of the nature of the correct solutions to the concept problems. New information was properly integrated through a substantive modification of the original hypothesis. However, this integration of new information was not remembered unless the "new" information was seen several times. Thus even this reduced memory condition was at the limits of this 96 year old's memory capacity.

Significance to Bio-Medical Research and the Program of the Institute:

Reasoning is among the most prized behaviors of man and among the most elusive for experimental study. In this project, methods have been and will be developed to obtain quantifiable measures of step-by-step performance on reasoning problems. Some of these methods also provide patterns of response which represent strategies in solving such problems.

Measures are obtained in current experiments to study changes in reasoning processes with age. These studies, in addition to identifying basic reasoning processes, should indicate the pervasiveness of reasoning deficits with age, whether education and cognitive activity mitigate such deficits, and what techniques could be used to minimize decline in reasoning.

Proposed Course of Project: Data collection will continue in Experiment V, the longitudinal study. Analyses of preliminary data will continue in order to determine the factors contributing to the age declines in effectiveness of solutions. Model construction will continue in Experiment XI. An attempt is being made to construct automated equipment which will allow for the timing of all aspects of the concept learning problems.

Publications: None

SMITHSONIAN GOV. INFO. EXCHANGE PROJECT NUMBER (DO NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  <div style="text-align: center; font-weight: bold;">Z01 AG 00065-17 LBS</div>
PERIOD COVERED <div style="text-align: center; font-weight: bold;">July 1, 1976 to September 30, 1977</div>		
TITLE OF PROJECT (80 characters or less)  <div style="text-align: center; font-weight: bold;">Verbal Learning and Age</div>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <div style="display: flex; justify-content: space-between;"> <div> <b>PI:</b> David Arenberg   <b>Other:</b> None         </div> <div style="text-align: center;"> <b>Chief, Learning &amp; Problem Solving Section</b> </div> <div style="text-align: right;"> <b>LBS GRC NIA</b> </div> </div>		
COOPERATING UNITS (if any) <div style="text-align: center;">           Baltimore City Hospitals            E. A. Robertson-Tchabo, University of Maryland         </div>		
LAB/BRANCH <div style="text-align: center;">Gerontology Research Center - Laboratory of Behavioral Sciences</div>		
SECTION <div style="text-align: center;">Learning and Problem Solving</div>		
INSTITUTE AND LOCATION <div style="text-align: center;">NIA, NIH, Baltimore, Md. 21224</div>		
TOTAL MAN-YEARS: <div style="text-align: center; font-weight: bold;">1.70</div>	PROFESSIONAL: <div style="text-align: center; font-weight: bold;">.30</div>	OTHER: <div style="text-align: center; font-weight: bold;">1.40</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div> <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS   <input type="checkbox"/> (a1) MINORS    <input type="checkbox"/> (a2) INTERVIEWS         </div> <div> <input type="checkbox"/> (b) HUMAN TISSUES         </div> <div> <input type="checkbox"/> (c) NEITHER         </div> </div>		
SUMMARY OF WORK (200 words or less - underline keywords)  <p>The primary purposes of this project on <u>aging</u> are to identify measures of <u>verbal learning</u> and <u>memory</u> which change with <u>age</u>, to specify <u>psychological processes</u> and their relationships with age, and to develop <u>procedures for improving learning and memory</u> performance in the elderly.</p>		



Project Description:

Objectives: Primary objectives are: (1) to identify which aspects of learning and memory change with age (and which do not); (2) to specify psychological processes underlying such age changes; (3) to identify health, nutrition, biochemical, and personality variables which are correlated with performance or with change in performance; and (4) to develop procedures to improve learning and memory performance in the elderly.

Methods Employed: Experiment XXXII is a study, in collaboration with the Clinical Physiology Branch, of the effects of propranolol on learning and information processing. Learning is measured by a multi-trial, free-recall procedure. In addition, simple reaction time is included along with four choice-reaction-time tasks in which the cognitive load is varied. Subjects receive an injection of propranolol or saline (double blind--neither the subject nor the experimenter knows what is injected). Several hours before and two hours after the injection, all performance measures and concurrent heart rate recordings are obtained. Two aspects of this study are: (1) the effects of propranolol on performance in young and old men; and (2) the relationship among heart rate, cognitive load, and performance in young and old men.

Experiment XXXIII is a new longitudinal study which includes immediate free recall, delayed memory, dichotic listening, forward digit span, simple reaction time, and four levels of choice reaction time. These procedures (somewhat modified) were included in Experiment XXXI of this project. In immediate free recall, twelve familiar words are presented visually at a rate of one per second, and the task is to recall as many words as possible (in any order and without time limits) at the end of the presentation. Two delayed memory procedures are included. In delayed recall, the task is to recall as many words of the free-recall list as possible after an interpolated task (dichotic digits). In delayed recognition, the twelve words in a free-recall list mixed with 12 other familiar words are presented in random order after the interpolated task; the recognition task is to identify which words are from the list and which are not. In dichotic listening, two different digits or two letters are presented simultaneously, one to each ear; the task is to identify the two stimuli. In forward digit span, lists of three to nine digits are presented at a rate of one digit per second, and the task is to respond with all the digits in a list in the order presented. In simple reaction time, a zero appears on a screen 15 times during a 90 second period, and the task is to press a button as fast as possible for each stimulus. In the four choice reaction procedures, 90 digits appear at a rate of one per second, and 15 of these digits are target stimuli which are signals to press the button as fast as possible. The four rules for responding are: (1) a specific digit; (2) any odd (or even) digit; (3) any even digit which immediately follows an odd digit (or vice versa); and (4) any even digit which immediately follows an even digit, and any odd digit which immediately follows an odd digit.

Major Findings: Experiment XXXII is the study of propranolol's effects on learning and information processing in young and old men. Preliminary

analyses of the multi-trial free-recall data were based upon pre-and post-infusion measures for 12 young (28-36 yrs) experimental subjects, 10 old (62-78 yrs) experimental subjects, and 6 old (61-80 yrs) control (saline rather than propranolol) subjects. Post-infusion means for total errors were higher (poorer performance) than pre-infusion means for all three groups. There is no support in these data for improved performance in learning from propranolol.

Experiment XXXIII is the longitudinal study of memory and information processing. Equipment has been constructed and new dichotic tapes are in the process of being prepared. Data collection is expected to begin by the beginning of the fiscal year.

Significance to Bio-Medical Research and the Program of the Institute: Learning is more central to experimental psychology than any other behavior, and some of the most striking and consistently reported behavioral age differences in the gerontological literature have been found in verbal learning performance. The experiments in this project are designed to identify basic mechanisms of learning and retention and to measure differences and changes in these functions that occur with age. In addition, knowledge about experimental variables which affect age differences will be valuable in developing techniques for optimizing learning of the older person.

Proposed Course of Project: Efforts continue to prepare dichotic digit tapes in which (1) stimulus onsets are perceived as simultaneous, and (2) intensities are perceived as equivalent. Dichotic letter tapes are also being prepared with the same goals. These tapes will not only be used together with the dichotic digit tapes in the longitudinal study, but will be used in a new study to explore the recent findings that some elderly men cannot attend to both of two simultaneous inputs (two different digits, one to each ear). Young men (and many old men) do not experience this difficulty. In the new study, onsets of letter pairs will be offset rather than simultaneous to determine the minimal asynchrony an old person (who cannot identify simultaneous pairs) requires to identify both letters. This separation time is an index of switching time required to attend to two inputs presented close together in time. Minimal switching will be related to the psychological refractory period, the time during which the second of two stimuli (tones) presented closely in time cannot be processed because the system is "refractory" due to the first tone (see Project Number Z01 AG 00066-16, Experiment VIII).

Publications:

Arenberg, D: The effects of input condition on free recall in young and old adults. Journal of Gerontology, 1976, 31: 551-555.

Arenberg, D. and Robertson-Tchabo, E. A.: Learning and Aging. In Birren, J. E. and Schaie, K. W. (Eds.): Handbook of the Psychology of Aging, New York, Van Nostrand, 1977, pp. 421-449.

- Arenberg, D. Memory and learning do decline late in life. In Proceedings of Vichy Conference, "Aging: A Challenge to Science and Social Policy." Pergamon Press, in press.
- Botwinick, J. and Arenberg, D.: Disparate time spans in sequential studies of aging. Experimental Aging Research, 1976, 2: 55-61.
- Robertson-Tchabo, E. A. and Arenberg, D.: Aging, Learning, and Problem Solving. In Wolman, B. B. (Ed.): International Encyclopedia of Psychiatry, Psychology, Psychoanalysis; Neurology. New York, Van Nostrand Reinhold/Aesculapius, 1977, Volume No. 1, pp. 357-359.
- Robertson-Tchabo, E. A. and Arenberg, D.: Age differences in cognition in healthy, educated men: A factor analysis of experimental measures. Experimental Aging Research, 1976, 2: 75-89.
- Robertson-Tchabo, E. A., Hausman, C. P., and Arenberg, D.: A classical mnemonic for older learners: A trip that works. Educational Gerontology, 1976, 1: 215-226.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00066-16 LBS
PERIOD COVERED July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (80 characters or less)  Perceptual Retention and Age		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  PI: David Arenberg Chief, Learning and LBS GRC NIA Problem Solving Section  OTHER: None		
COOPERATING UNITS (if any) Baltimore City Hospitals W. W. Surwillo, University of Louisville		
LAB/BRANCH Gerontology Research Center - Laboratory of Behavioral Sciences		
SECTION Learning & Problem Solving		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Md. 21224		
TOTAL MANYEARS: 1.55	PROFESSIONAL: .50	OTHER: 1.05
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The primary purposes of this project on <u>aging</u> are: (1) to investigate <u>percep- tual retention and interference</u> ; (2) to determine under what conditions age differences in retention are affected by interference; and (3) to investigate processes of interference and perception. Studies involve <u>psychologicl refractory period and non-verbal memory</u> . A <u>longitudinal study of personality</u> , using the <u>Guilford-Zimmerman Temperament Survey</u> , analyzed changes within subjects and included <u>cross-sectional, cross-sequential and time-sequential</u> effects. Results indicated <u>maturational changes, cultural (secular) changes,</u> and <u>generational differences</u> .		

Project Description:

Objectives: One general objective is to investigate the effects of interference in perceptual retention and in perception: (1) to determine whether aging results in increased susceptibility to interference; (2) to explore conditions which affect age differences in interference; and (3) to develop procedures for testing mechanisms which may account for the empirical findings. Another objective is to study non-verbal memory and the conditions which improve such memory, especially for the old.

Methods Employed: Experiment VIII is a study of age and the psychological refractory period. When two signals are presented close together in time and the task is to respond to the second, the reaction time increases as the interval between signals decreases (over the range of 500 to 50 msc). The additional time required is known as the psychological refractory period. One explanation is based on a model involving a single channel of limited capacity which processes information sequentially. When two signals occur successively at a fast rate, the system cannot attend to and process both. As a result, the second signal must be stored until the previous signal has been processed and the system is no longer refractory. This experiment was initiated to test the hypothesis that the psychological refractory period increases with age. Pairs of auditory signals are presented at interstimulus intervals of 50, 100, 250, 500, 750, and 1,000 msc. If the psychological refractory period increases with age, intervals 100 msc and longer which do not affect response times of young men should affect response times of older men.

Experiment IX is a longitudinal study of personality based on data from the Guilford-Zimmerman Temperament Survey (GZTS). The GZTS is a questionnaire which measures ten personality traits. Data collection began in 1958 and is continuing at the present time. Current analyses are based on data collected from 1958 through mid-1974 on men ranging in age from 17 to 98 ( $N = 915$ ). For several analyses the sample was dichotomized into men who entered the study early (1958 to mid-1968,  $N = 605$ ) and those who entered the study later (mid-1968 to mid-1974,  $N = 310$ ). Repeated measures were obtained after an interval of approximately 7 years for 336 men in the early sample. In addition to cross-sectional and longitudinal (repeated-measures) analyses, cross-sequential and time-sequential analyses were carried out. Cross-sequential analyses provide a replication of longitudinal findings while eliminating biases which may result from repeating the measure, and also are less affected by sample attrition. Time-sequential analyses provide a comparison of men who are the same ages but were tested at different times; such analyses are useful in trying to separate cultural effects from maturational effects.

Major Findings: Experiment VIII is the study of age and the psychological refractory period. Preliminary results indicate that for older men response time is increased over a wider range of intervals between two auditory stimuli than for young men. This result is consistent with two models of the

psychological refractory period: (1) the refractory period increases with age; and (2) the refractory period does not change with age, but the range of interstimulus intervals during which the system is refractory is extended with age. The current results are more consistent with the latter. As the interval decreases from 1,000 to 100 msc, an older man seems more likely to be refractory when the second stimulus occurs than a young man. At the 50 msc interval, where the refractory effect should be greatest for everyone, the difference between the men below 35 and the men over 70 is not much larger than the difference between those two groups at the long interstimulus intervals where the refractory effect should be least. If the refractory period increases with age, age differences should be substantially larger at 50 msc than at the long interstimulus intervals.

Experiment IX is the longitudinal study of personality. It was found that five of the ten measures of the Guilford-Zimmerman Temperament Survey declined longitudinally with age, but only two scales showed declines which were consistent with a maturational interpretation. These two scales are called General Activity and Masculinity.

General Activity predominantly involves the pace of a person's activity. A high score characterizes an energetic, rapid-moving, rapid-working person who likes action and may sometimes be impulsive. This scale declined, but the change varied according to age group. General Activity scores actually increased somewhat for the men initially in their twenties, and only the means for the groups over fifty declined.

Masculinity, on the other hand, declined consistently across the age span. This scale is a composite dimension predominantly involving so-called "masculine" interests. A high score characterizes a person who is comfortable with guns and hunting and who has little compassion for animals, does not cry or express emotion easily, and is not given to ready feelings of fear or disgust.

The other three scales which declined longitudinally are called Thoughtfulness, Personal Relations, and Friendliness; but these declines are not interpretable as maturational changes. The declines in Thoughtfulness and Personal Relations appear to be cultural changes specific to the two times of measurement. This interpretation is based on the findings that (1) initial measures of men who joined the study late were lower on these two scales than initial measures of men of comparable ages who entered the study early, and (2) cross-sectionally (at a specific time), no age differences were found. The Thoughtfulness scale measures reflectiveness. A high score characterizes an introspective, meditative person who is given to analytic and evaluative thinking and ponders over the past. Personal Relations is a scale measuring tolerance and cooperativeness. A high score characterizes a trustful person who thinks well of people and of institutions and who is not given to fault-finding or self-pity.

The longitudinal declines in Friendliness are consistent with a long-term cultural decline. In addition to the differences between groups of men of

the same age who were measured early and late in the study, cross-sectional differences showed increases with age. Friendliness is a scale with agreeableness at one extreme and hostility at the other. A high score characterizes a compliant person who is not contemptuous of others, does not resent being given orders, and is not easily aroused to belligerence or aggressive behavior.

Cross-sectional age differences were found for two other scales, but longitudinal changes were not found. Restraint increased with age, and Ascendancy decreased with age. The data for these scales were consistent with generation differences rather than maturational changes.

These findings demonstrate how misleading cross-sectional age differences can be about age changes and how even longitudinal changes based on repeated measures require supporting data to permit conclusions about maturational changes.

Significance to Bio-Medical Research and the Program of the Institute: The general idea that a person becomes more susceptible to interference as he grows older is well entrenched in gerontological thinking and is often used to "explain" age differences in performance. The evidence for this idea, however, is sparse and not consistent. It is the purpose of this project to explore the generality of the age-interference hypothesis for non-verbal memory and perception. It is important, both for theoretical and applied reasons, to identify those conditions which are especially interfering for the old. Furthermore, correlative studies of interference behavior and physiological variables, such as the alpha period of the EEG, should improve our understanding of the processes underlying the behavior.

Proposed Course of Project: If the preliminary results of the study of the psychological refractory period hold up, an additional study with several interstimulus intervals between 100 and 500 msc (rather than one) would be warranted to provide additional information to understand better the phenomenon and how it is related to age. Such additional data would also provide indices of individual refractoriness which could be related to "switching" time in the study of asynchronous dichotic listening proposed in Project Number Z01 AG 00065-17 LBS. The longitudinal study of personality will be continued to determine whether those changes which were interpreted as maturational and those interpreted as cultural are confirmed by longer-term and additional repeat data.

#### Publications:

Arenberg, D. The effects of auditory augmentation on visual retention for young and old adults. Journal of Gerontology, 1977, 32: 192-195.

Arenberg, D. Differences and changes with age in the Benton Visual Retention Test. Journal of Gerontology, in press.

Arenberg, D. and Thorne, P. Are channels relevant to order of recall?  
Yes. Psychological Reports, 1976, 38: 271-274.

Douglas, K. and Arenberg, D. Age changes, cohort differences, and cultural change on the Guilford-Zimmerman Temperament Survey. Journal of Gerontology, in press.



PROJECT NUMBER (Do NOT use this space)

HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

Z01 AG 00067-10 LBS

## PERIOD COVERED

July 1 1976 to September 30, 1977

## TITLE OF PROJECT (80 characters or less)

Learned Modification of Visceral Function in Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER  
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. T. Engel	Chief, Lab. of Behavioral Sciences	GRC, NIA
Other:	W. F. Baile	Staff Fellow	LBS, GRC, NIA
	J. H. McCroskery	Visiting Scientist	LBS, GRC, NIA
	E. G. Lakatta	Chief, CV	CPB, GRC, NIA
	M. K. Garwood	Staff Fellow	LBS, GRC, NIA

## COOPERATING UNITS (if any)

G. C. Voigt, Cardiovascular Division, BCH  
S. H. Gottlieb, Cardiovascular Med., Johns Hopkins School of Medicine  
W. G. Reiner, Dept. of Urology, Johns Hopkins School of Medicine

## LAB/BRANCH

Laboratory of Behavioral Sciences

## S.

Psychophysiology

## INSTITUTE AND LOCATION

GRC, NIA, NIH, Baltimore, Maryland 21224

## TOTAL MANYEARS:

5.00

## PROFESSIONAL:

3.50

## OTHER:

1.50

## CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS☐ (b) HUMAN TISSUES☐ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

## SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with the interaction of behavior and physiology in man. One study investigates the application of operant conditioning (biofeedback) in heart rate control by patients with angina pectoris. A second study considers the application of operant conditioning to patients with urinary incontinence. A third study explores age differences in the physiological mechanisms underlying electrodermal activity. A fourth study investigates the application of behavioral techniques in patients with myocardial infarctions to see if these can facilitate compliance in these patients.

Project DescriptionObjectives

A. 1. To see if patients with angina pectoris can learn to slow their heart rate upon signal after training with operant conditioning procedures.

2. To see if such training will enable patients with angina pectoris: (a) to lower their heart rate while engaging in mild activity; (b) to lower their heart rates while engaging in strenuous activity (exercise stress test) such that the rate-pressure product will be lower and exercise duration will be longer; and (c) to decrease the number of anginal episodes while going about their daily routines.

B. To determine whether patients with urinary incontinence can be trained to become continent.

C. To determine if there are age differences in the electrical properties of the skin. Age differences in the effect of manipulating the parameters of the skin specified by models of electrodermal activity suggest that there are age-related alterations in the relative magnitudes of the sweat gland and epidermal potentials. Current research focuses on age differences in the relationship between epidermal hydration and electrodermal activity.

D. To facilitate compliance with a therapeutic regimen in a population of survivors of myocardial infarction (MI). The patients were all considered to be at risk for breaking a recommended activity regimen during their convalescence. This non compliance was medically worrisome because it could result in reinfarction or other cardiovascular complications (e.g., sudden death from arrhythmia) .

Methods Employed

A. 1. The training to lower heart rate upon signal uses operant conditioning procedures and apparatus that have been developed in this laboratory. The patient reclines on a hospital bed watching a panel of lights and a meter at the foot of the bed. After a 25-min. adaptation period during which a 10-min. baseline heart rate is taken, the patient receives a 17-min. training period. A red light on the panel signals the patient to lower heart rate and a yellow light comes on to inform the patient whenever he has reduced his heart rate below the criterion level. The meter informs the patient of percent time correct.

As the patient begins to show ability to lower heart rate, sessions in which alternating periods of feedback and no-feedback are introduced so as to give the patient training in heart rate control independent of the light and meter feedback. Initially these alternate sessions consist of one minute feedback alternating with one minute of no-feedback, but then ratios of 1:3 and then 1:7 are introduced such that on the last sessions the patient has a

little over 2 minutes of feedback and a little over 14 minutes of no-feedback.

2. There are three measures taken before and after training to indicate change.

a. Each patient daily fills out a short questionnaire which describes any anginal episodes that may have occurred. These self-reports provide information about changes from pre-training to training to post-training in daily routine.

b. Four times during the month of pre-training, after every third training session and once during the month of post-training the patient participates in a course walk. He walks an 81-m distance while his heart rate is recorded on a Holter monitor. This data will show if the heart rate acquired in the laboratory will transfer to mild activity.

c. Twice during pretraining and once post-training the patient exercises on a treadmill. The treadmill revolves at a constant speed but is elevated every three minutes. Heart rate is recorded via an electrocardiogram and blood pressure is taken at regular intervals. The patient exercises until he reports chest discomfort and/or until there are electrocardiographic changes depicting ischemia. These measures will show the effect of heart rate control during strenuous activity.

B. Patients with documented, clinically significant urinary incontinence secondary to the following conditions are eligible: cerebral vascular accidents, spinal cord trauma, tumor or malformation, surgery, diabetic neuropathy, and demyelination of the nervous system. The hypothesis to be tested is that operant conditioning (biofeedback) will enable learning of appropriate bladder and sphincter control so that the degree of incontinence will be markedly reduced. The experimental design will be a simple before-and-after method with each patient serving as his own control. The procedure is simplified in that the diagnostic techniques are coordinated with the training procedures.

There are essentially three phases to the proposed study. Phase I is a diagnostic procedure during which the nature and severity of the bladder and sphincter control will be determined. First there will be an interview to obtain a record of the degree of incontinence in the daily routine. Then by means of CO<sub>2</sub> cystometry, pressure in the bladder and along the urethra will be obtained. The procedure involves inserting a catheter through the urethra into the bladder and an EMG needle through the perineum into the external sphincter for muscle recording. The catheter has two small openings (one at the tip and another 4 cm. back from the tip) for pressure recording. Electrical outputs from the transducers will be amplified and recorded by a direct-writing polygraph. Controlled amounts of CO<sub>2</sub> will be introduced into the bladder through the catheter by means of a regulator to give a filling pressure. Before training intra-vesical and intra-urethral pressures will be taken. These will provide a diagnosis of the nature of the incontinence and provide remedial guidelines for that patient.

Phase 2 is the initial training procedure. Training will consist of showing the patient the immediate record of the sphincter responses as they appear on the polygraph for comparison with tracings of normal sphincter responses. In addition to visual feedback the patient will be given verbal praise for successful responding and encouragement to continue modifying these responses.

Phase 3 will consist of additional training to aid the patient in developing a strong response and in learning to respond independently of the polygraph. As the patient begins to show appropriate sphincter contractions, the filling pressure will be gradually raised so as to require a stronger sphincter response. As the patient learns to respond to normal filling pressures, feedback will be periodically withheld by covering the polygraph so that the patient can not see the tracing.

Each training session will last approximately two hours and will be spaced at weekly intervals. The patient will be urged to practice sphincter control between sessions. Since it is a major intention to develop a quick procedure, no patient will receive more than five sessions. One month after the last session the patient will return to the laboratory for a follow-up session to assess retention of the sphincter control. At this time a second interview will be conducted to determine the degree of improvement in continence.

C. Electrodermal activity is recorded on a Beckman recorder. Epidermal hydration is varied by use of different electrolytes and procedures. The condition of least hydration uses a .5% glycol electrolyte. Two additional conditions use a .5% aqueous electrolyte differing in that the condition of most hydration receives a 15 minute presoak in distilled water whereas the condition of intermediate hydration receives no pretreatment. These three hydration conditions are used in recording 3 channels of skin potential and 3 channels of skin resistance from palmar surfaces. Subjects are tested under three conditions that produce successive increments in sweat gland activity; (1) tone presentations after test, (2) simple reaction time, and (3) choice reaction time. Subjects are participants in the Baltimore Longitudinal Studies.

D. Subjects were nine middle-aged men hospitalized in a Coronary Care Unit at the time of the study. They were chosen from a group of patients referred by the staff physician or staff nurse who had judged them to be at high risk for non-compliance during the post-MI period. Our selection was based on the findings from an evaluation interview. All patients who were selected for this study met one or more of the following criteria; 1) past non-compliance with a similar regimen (N=2); 2) unrealistic expectation of post-infarction work ability (N=3); 3) over-investment in a life-style (work-style) for which a slowing down of activity would be an obstacle to rehabilitation (N=3); 4) actual non-compliance in the CCU (N=3); 5) denial of infarct (expression of doubt as to the diagnosis) (N=4); 6) extreme delay behavior in getting to the hospital (N=2); 7) statement as to unwillingness to comply with the rehabilitation program (N=1). Seven patients agreed to

participate in the program. The two who declined continued to behave in the post-infarction period as though they had not had a serious heart attack.

Each patient was told in some detail about the specific nature of his infarct, and he was shown his enzyme studies and EKG so that he could have a good insight into the nature of his MI. The patient then was requested to plan a hierarchical program of rehabilitation which was appropriate for him. Each program had the following features: 1) There were 9 or 10 steps; 2) The last step was full-recovery as defined by the patient (for most patients this was "return to full-time work"); 3) each step in the hierarchy was more difficult (i.e., required a greater number of metabolic equivalent units) than the prior step; 4) every patient was taught to take his pulse and was supplied with forms to record his pulse before and after each activity as well as his symptoms at each level. Patients who began the program in the hospital (N=6) were seen daily and then weekly on an out-patient basis. When appropriate, activity lists were modified to add new activities. Pulses were checked periodically by the investigator. Criteria for movement to the next level of activity included: (1) a pulse rate change during the activity of 20 bpm or less from baseline; (2) pulse rate <110 bpm for a particular activity; (3) absence of symptoms for a particular level of activity. Results were analyzed in terms of: (1) number of appointments kept per patient; (2) average number pulse recordings/day over time; (3) number of times patient went out of activity level as reported by wife or patient himself; (4) number of appointments kept in clinic; (5) consistency of pulse changes with different activities; (6) comments of patient's clinic physicians.

### Major Findings

A. Six patients have entered the study thus far and have continued until the first exercise test. Only two patients have provided sufficient data to continue into the training phase of the study. Two patients were discontinued due to adequate but negative tests, one was discontinued because of unreliable reports of chest pain and one was discontinued due to the development of an irregular rhythm. The study is still in progress. Information about the one patient who had completed the program and one who is in the middle of training is presented below.

1. C.B. received 18 training sessions in heart rate slowing. By the last session he produced an 8 bpm decrease in heart rate upon signal and maintained this over 14 minutes of no-feedback. On the course walk his rate during the last third of the walk after training was 9 bpm lower than the pretraining average and 11 bpm lower on post-training; pre-walk measures were also lower after training began. On the exercise test he continued for 3 min longer and reached a higher grade elevation on the post-training test; however, his heart rate was not different and his blood pressure rose faster. The daily self-report data was not helpful in that he had few anginal episodes.

2. M.B. has had six heart rate training sessions. On the last session he produced a 7 bpm decrease upon signal with and without feedback. On the two training course walks he has achieved rates over the last third of the

course that are 5 bpm lower than the pre-training average.

B. The study is at present in an initial phase. Experience with a first patient has suggested some limitations on the ability to be trained, i.e., individuals lacking an anatomically intact sphincter mechanism will probably maintain poor control. On the other hand, one biofeedback session with a second patient who was suffering from incontinence secondary to a cerebral vascular accident successfully developed increased sensation of bladder filling, and inhibited involuntary emptying long enough for him to use the toilet.

C. For young adults the lowest skin conductance level occurred with least hydration; however, there was not a monotonic relationship between skin conductance level and hydration. This result is consistent with previous findings and has been explained on the basis of two mechanisms. Increasing water content of the epidermis tends to increase skin conductance level because increased water will allow more current through the skin. However, at high levels of hydration, mechanical obstruction of the sweat duct occurs tending to lower skin conductance level. Therefore, hydration should show some relationship with skin conductance level, but the relationship should not always be monotonic because of the influence of opposing forces. Aged subjects also showed the lowest skin conductance level with least hydration, but additionally showed a monotonic relationship between hydration and skin conductance level. One explanation for this result is that dryer aged epidermis can tolerate more hydration before opposing effects of sweat duct obstruction occur.

For young subjects, skin potential level was monotonically related to hydration with more negative skin potential levels occurring with less hydration. This finding is consistent with previous research and models of the mechanisms of electrodermal activity. In their simplest form these models specify two sources of potential (sweat gland and epidermal potentials), and two sources of resistance (sweat gland and epidermal resistances). The potential sources are arranged in parallel with the sweat gland potential larger than the epidermal potential. When epidermal resistance is low as in hydration, current is drawn through the epidermis lowering the recorded voltage. Results for the aged were quite different. The least negative skin potential level occurred with least hydration. We postulate that this reversal in hydration/skin potential level relationship in old age reflects a reversal in relative magnitudes of sweat gland and epidermal potentials: in old subjects epidermal potential is greater than sweat gland potential whereas, in young subjects sweat gland potential is greater than epidermal potential.

D. Data has been completely collected on five patients and will soon be analyzed for the last two. Preliminary indications show the following results: (1) compliance with appointment keeping of nearly 100%; (2) adherence to the prescribed level of activity approximately 85% of the time; (3) completeness of pulse recording 90% of the time; (4) death in two patients from reinfarction; (5) return of three patients to full work activity;

(6) elimination or reduction of several unwanted behavioral characteristics by several patients.

#### Significance to Bio-medical Research and the Program at the Institute

A. Although the findings to date are very tentative, they are suggestive. Both patients have demonstrated heart rate control and it appears that this control can transfer to a mildly active task. Should these findings be supported by the remaining patients it would be the first report on the effects of operant conditioning in patients with angina pectoris and provide a new training procedure to supplement those now being used.

B. Urinary incontinence is a malady which accounts for a large percentage of admissions of otherwise functional people to geriatric wards. Apart from the social consequences, which may range from early institutionalization to severe disruption of lifestyle, medical complications may include skin eruptions or erosions, decubital ulcers or shrinkage of the bladder. Aside from having the potential to provide a treatment modality for the elderly who are most frequently afflicted by this problem, this project will hopefully provide further data in the area of operant conditioning of visceral function in man.

C. Studies of the physiological mechanisms involved in electrodermal activity will permit us to understand in greater detail how the nervous system controls peripheral sudomotor responses, and how age changes express themselves--i.e., in the effector, in the nervous system or in both. This research also has potential clinical value. Some of the most dramatic changes in aging occur in the skin, and there are a number of diseases which occur in older subjects that are related to changes in the properties of the skin. It may be possible to diagnose or to predict the course of these disorders more precisely (or of therapeutic interventions) through a clearer understanding of electrical properties of the skin and the mechanisms which regulate these properties.

D. Longevity after myocardial infarction is in part a function of the patients willingness to comply with a sensible activity program. Impatience, time-urgency and speed of action have been shown to characterize many MI patients and to interfere with their rehabilitation. This program by: (1) providing concrete goals; (2) focusing on a gradual increase in habitual activities; (3) promoting sensible and beneficial exercise, can make this period less difficult and prevent further cardiac damage and psychological upheaval.

#### Proposed Course

A. Continue the project.

B. Recruitment and training of subjects will continue. More refined observational techniques to document and record the degree of the incontinence prior to training will be included.

C. Research in progress involves determining whether age differences in epidermal hydration are due to alteration in relative magnitude of sweat gland and epidermal potentials. This is currently being assessed by manipulating the magnitude of epidermal potential through use of electrolytes with different salt concentrations. Another study involves relating sweat rate and sweat sodium concentration to different aspects of electrodermal activity to determine whether the same mechanisms are reflected in electrodermal activity of young and old people.

D. Data will be complete for the two patients in the near future. A new project will be initiated to investigate the demographic, psychological and medical characteristics of patients who leave from the CCU against medical advice. A project under contemplation will investigate prediction of non-compliance in CCU patients. The treatment compliance program we have initiated will be maintained on the CCU.

#### Publications

Baile, W. F. and Brinker, J. A.: Death from fright. Letter to Editor, Psychosom. Med. 39(3): 198-199, 1977.

Engel, B. T.: Cardiac Arrhythmias. In Williams, R. B., Jr. and Gentry, W. D. (Eds.): Behavioral Approaches to Medical Practice. Cambridge, Ballinger Publishing Co., 1977, 67-76.

Engel, B. T.: Fecal Incontinence. In Williams, R. B., Jr. and Gentry, W. D. (Eds.): Behavioral Approaches to Medical Practice. Cambridge, Ballinger Publishing Co., 1977, 167-172.

Engel, B. T.: Biofeedback as treatment for cardiovascular disorders: a critical review. In Beatty, J. and Legewie, H. (Eds.): Proceedings of NATO Symposium on Biofeedback and Behavior. Munich, Germany, Plenum Press, 1977, 395-401.

Weiss, T. and Engel, B. T.: Modification of psychosomatic behaviors. In Davidson, R. S. (Ed.) Experimental Analyses of Clinical Phenomena, in press.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00068-15 LBS
PERIOD COVERED <p style="text-align: center;">July 1, 1976 to September 30, 1977</p>		
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Operant Performance, Memory and Aging</p>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <div style="display: flex; justify-content: space-between;"> <div>           PI: Charles L. Goodrick            OTHERS: None         </div> <div>           Research Psychologist    </div> <div>           LBS GRC NIA         </div> </div>		
COOPERATING UNITS (if any)  <p style="text-align: center;">Baltimore City Hospitals</p>		
LAB/BRANCH <p style="text-align: center;">Gerontology Research Center - Laboratory of Behavioral Sciences</p>		
S. Learning & Problem Solving		
INSTITUTE AND LOCATION <p style="text-align: center;">NIA, NIH, Baltimore, Md. 21224</p>		
TOTAL MANYEARS: <p style="text-align: center;">.20</p>	PROFESSIONAL: <p style="text-align: center;">.10</p>	OTHER: <p style="text-align: center;">.10</p>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div> <input type="checkbox"/> (a) HUMAN SUBJECTS   <input type="checkbox"/> (a1) MINORS    <input type="checkbox"/> (a2) INTERVIEWS         </div> <div> <input type="checkbox"/> (b) HUMAN TISSUES         </div> <div> <input checked="" type="checkbox"/> (c) NEITHER         </div> </div>		
SUMMARY OF WORK (200 words or less - underline keywords) <p>           The major purpose of this project is to analyze <u>complex maze learning</u> of young and aged animals, and to determine techniques which act to improve learning ability. Another goal of this project is to determine age differences in <u>operant performance</u> for young and aged <u>rats</u> or <u>mice</u>, and to determine factors which may act to improve performance, and also improve <u>retention</u> of the learned responses.         </p>		

Project Descriptions:

Objectives: The general objectives of one phase of this project are: (1) to analyze complex maze learning of young and aged animals; and (2) to determine variables which may act to enhance or retard maze learning ability. The objectives of the other phase of this project are: (1) to study age differences in motor performance during operant responding; and (2) to develop operant techniques which improve the retention of learned responses in aged animals.

Methods Employed: Operant conditioning performance and retention studies have used 2-bar test boxes in which hungry animals are trained to press one bar to obtain a food reward while the alternate bar remains neutral. By increasing the complexity of the task (using two bars rather than one), it is possible to make a finer analysis of performance and the retention process. We are studying performance and retention as a function of reward schedule, and we are particularly interested in the partial reinforcement effect. The retention of partially rewarded responses is vastly greater than responses continuously rewarded; and analysis of this phenomenon will provide information regarding the general retention process.

A complex 14-unit multiple-T maze also is utilized. This maze has been shown to be a highly reliable test of learning, and it has been used in many major studies of aging. Additional mazes of 6-units are being developed to study mastery of consecutive problems by young and aged rats. These mazes will be used to analyze aging effects in short-term and long-term memory and to determine aging effects in interference, both proactive and retroactive.

Major Findings: Data are currently being analyzed. They will be reported in detail in next year's annual report.

Significance to Bio-Medical Research and the Program of the Institute: Learning and/or memory deficits represent a major problem among the aged human population. Major behavioral techniques to reduce performance deficits obtained for aged animals have been studied in our laboratory. This project may facilitate research with man by identifying optimal conditions for learning and for retention of learned responses.

Proposed Course of Project: Further studies are in progress to determine the nature of the partial reinforcement effect in relation to: (a) time contingent vs. response contingent partial reinforcement; and (b) massed vs. distributed extinction trials. Other studies will examine age differences in operant performance as a function of response effortfulness. Maze studies will concentrate upon the effects of central nervous system stimulants on behavioral rigidity within the maze for old rats. We will also initiate preliminary studies of perceptual learning in humans to determine the generality of the massed practice effects found for aged rats.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF          INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER  Z01 AG 00069-12 LBS
PERIOD COVERED      July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (80 characters or less)  Exercise, General Activity Level and Aging		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  PI:                Charles L. Goodrick      Research Psychologist      LBS GRC NIA OTHERS:        None		
COOPERATING UNITS (if any)  Baltimore City Hospitals		
LAB/BRANCH Gerontology Research Center    LBS		
SC                Learning and Problem Solving		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Md. 21224		
TOTAL MANYEARS: .80	PROFESSIONAL: .40	OTHER: .40
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  The major goal of this project is to determine the effect of voluntary <u>wheel</u> <u>exercise</u> upon <u>behavior</u> for animals tested in <u>lifespan</u> developmental research, and in addition to consider the effect of voluntary wheel exercise upon <u>longevity</u> . Another goal of this research is to increase the period of vigorous <u>activity</u> during later stages in the lifespan of the <u>rat</u> or <u>mouse</u> .		

Project Description:

Objectives: The general objectives are: (1) to determine methods for increasing vigorous physical activity of lower animals during late stages in the life span; (2) to examine behavioral and longevity differences among animals which differ in physical activity level; and (3) to determine the physiological mechanisms underlying differences in activity.

Methods Employed: Wistar rats or various strains of mice are placed in standard activity wheels and allowed access to free voluntary exercise. Hungry animals also may be rewarded with food for running. A technique to control experimentally body weight and to increase voluntary exercise utilizes feeding of animals on a periodic schedule, such as every other day. Other studies utilize inbred, hybrid, and mutant mice or species which differ in activity level due to different genetic constitutions (See Project Z01 AG 00061-15 LBS Behavioral genetics and aging).

Major Findings:

A. An investigation of voluntary wheel exercise and life span has been completed. In this study paired male and paired female Wistar rats ( $N = 140$ ) were maintained in cages with attached activity wheels (experimental groups) or in cages without access to activity wheels (control groups). The 40 males (Group 1,  $N = 12$ ; Group 2,  $N = 28$ ) and 40 females (Group 1,  $N = 12$ ; Group 2,  $N = 28$ ) allowed access to activity wheels were started under these conditions when 45 days old. The two experimental groups were started six months apart. Control pairs of 28 males and 32 females were 45 days old at the same time as experimental Group 1 male and female rats. Group 1 rats were started in July, 1973, while Group 2 rats were started in January, 1974. The mean life span of the male control group was 20.7 months, while the mean life span of the male experimental groups was 24.6 months. The difference in life span for the male experimental and control groups was highly statistically significant,  $t(66) = 4.28$ ,  $p < .001$ . The mean life span of the female control group was 26.2 months, with the mean life span of the female experimental groups 29.2 months, also a statistically significant difference,  $t(70) = 2.52$ ,  $p < .01$ . These differences provide strong evidence that voluntary exercise significantly increases life span.

An additional major effect occurred with respect to sex differences in life span. These sex differences in life span typically obtained in earlier studies were obtained in the present study for rat groups allowed voluntary wheel exercise as well as for controls (male vs. female, voluntary wheel exercise,  $t(78) = 4.30$ ,  $p < .001$ ; control  $t(58) = 5.08$ ,  $p < .001$ ).

The voluntary exercise groups also were found to have more long lived survivors than control groups, indicating that the significant differences in longevity were not due to higher mortality of the very young in the control groups than in the experimental groups. The longest lived survivors in the experimental groups were 33 months old for males and 39 months old for females, while the longest lived survivors of the control groups were 29 months old for males and 34.5 months old for females.

Body weight of control male rats increased until 12-15 months of age then decreased until death, while the body weight of control female rats increased until 18-21 months of age and then decreased until death. The body weight of experimental males increased until 15-18 months of age, then decreased until death, with the body weight of experimental females often increasing continually throughout the life span. These results suggest that duration of body weight increment may be related to group differences in longevity. Another factor was that body weights were significantly lower throughout the life span for experimental than for control groups and for female than for male groups. Analyses of the relations of body weight increment, peak body weight, and longevity will be completed at a later time.

B. In an attempt to increase voluntary wheel exercise later in the life span, additional pairs of male rats have been either allowed voluntary wheel exercise or placed as controls in normal cages, with food being restricted to every other day. Three groups of 12 experimental rats (N = 36) and three groups of 12 control rats (N = 36) are now being tested. The preliminary results are promising. The oldest groups (12 experimental and 12 control rats) are now 21 months old. No deaths have occurred for either control or experimental male rats, and voluntary exercise is considerably above that obtained at this age for rats fed ad libitum.

Significance to Bio-Medical Research and the Program of the Institute: One of the consistent findings of gerontological research is the decline in general activity level of old animals compared with young animals. It is important to determine whether quantity of activity (e.g., wheel activity) and/or quality of activity (e.g., increased exploration behavior or greater response variability) may be increased experimentally for old and senescent animals. It is also important to examine the role of heredity with respect to voluntary exercise throughout the entire lifespan, and the effect of exercise upon behavioral decrements associated with advanced old age. The knowledge and utilization of factors which change base activity levels of aged animals may result in the development of methods which can increase the productive later years of aged humans.

Proposed course of the project: The studies of rat wheel exercise will continue to determine the effects of voluntary exercise upon longevity for paired rats, and to determine the amount of voluntary exercise during advanced old age. Additional studies will determine the effect of reducing food intake by restricted every other day feeding upon voluntary wheel exercise and longevity. Also, more control groups will be added to obtain continuing data with respect to normal longevity.

Studies of wheel exercise periodicity of young and aged mice will be continued. Periodicity patterns of mice will be examined throughout old age. Additional studies will determine the level of voluntary activity for young and aged mice which are allowed voluntary control of lighting conditions within the home environment. Studies of the effect of dietary protein changes late in the life span upon voluntary wheel exercise and longevity have also been initiated using inbred mice.

Publications:

- Wax, T. Age, strain, and illumination intensity effects on behavior:  
I. Self-selection of locomotor activity and of light-dark schedules.  
Journal of Comparative and Physiological Psychology, 1977, 91: 51-62.

NIA Annual Report  
July 1, 1976 through September 30, 1977  
Gerontology Research Center  
Laboratory of Cellular and Comparative Physiology

This laboratory carries out studies to establish the cellular and molecular basis of aging. In addition, efforts are made to determine methods to eliminate or delay its deleterious effects.

One series of studies attempts to establish the effect of age on genetic expression. It appears possible that much of the aging process (the increased frequency of diseases and physiological dysfunctions) could result from cells slowly losing their normal highly specialized differentiated state. This would be manifested by either derepression and/or repression of genes that are not normally expressed or essential for cell function. Such alterations could clearly lower the operating efficiency of a cell and could even transform cells leading to certain types of cancer. Therefore the possible derepression of globin and C-type virus genes in brain and liver cells as a function of increasing age in mice was investigated. The results show up to a two-fold increase in the transcriptional expression of these genes with increasing age.

Another study was initiated to characterize the physico-chemical properties of the chromatin taken from specific cell populations of cells of animals throughout their lifespan. This information is necessary to determine if alterations of the chromatin could play an important role in underlining the general aging process of the animal. The results indicate that significant changes in the binding properties of chromatin proteins to DNA do occur with increasing age in mice. However, reports from other laboratories that a significant amount of single-stranded DNA also accumulates with age were not confirmed.

Cellular replication and DNA repair are of utmost importance to maintain normal cellular and organ function. The major objectives of another series of studies are to examine cell replication and repair of DNA damage as a function of aging in human cells in vitro and in rat and mouse tissues in vivo. Evidence for a loss of replicative ability with in vivo aging has been demonstrated during the past year. A technique has been developed to examine cell replication in vivo in intact animals utilizing the BrdU-differential staining technique. Preliminary results obtained with this technique indicate a decline in cell replication as a function of aging. Further research will be directed not only at defining the decline in cell replication but also at investigating the mechanisms for this functional loss.

The BrdU-differential staining techniques have also been applied to examining DNA repair by analyzing the frequencies of sister chromatid exchanges (SCE) induced by various DNA damaging agents (i.e., mitomycin C) (MMC) in vivo in mouse and rat bone marrow cells and in vitro in cultured human fibroblasts. In all these systems, a significant decrease in MMC-induced SCE was observed in the older cell populations. The mechanism for the altered response to DNA damage which is manifested by diminished SCE will be investigated.

The immune system is among the most important ones necessary for the maintenance of health. The decline in immunological responsiveness with age is well established but the reasons are not at all understood. The role of regulatory mechanisms in explaining the phenomena of immunosenescence may be of considerable significance.

Lymphoid cells from the spleens of old mice demonstrate poor cell division in several mitogen assay systems. The poor response can be explained by a decrease in the number of responsible cells, defective daughter cells which will not divide, and the presence of inhibitors which interfere with division. All these factors can be seen as causes of decreased cellular function in old mice. Furthermore, the immune memory of T-cells in mice is relatively short-lived so that secondary responses are lost in a year's time or less. Loss of memory, plus a decrease in responsiveness of T-cells seen with age may explain the age-related defect in cell-mediated immunity.

Identification of cell types has been facilitated by the development of cellular adaptation culture techniques and newer techniques for labeling antibody protein with fluorescent dyes.

The regulatory role of lymphoid cells and their products on the humoral immune response is the intent of another project. The regulatory mechanisms are being investigated in vitro using a T-cell dependent as well as a T-cell independent antigen. The cellular requirements and functions involved in the in vitro immune response are being established for normal adult mice. These are then being compared to those in aged mice of the same strains. The possibility that regulatory mechanisms observed in young adult mice are amplified in old mice resulting in immunosenescence is being investigated.

Nutrition has been the only environmental factor which has consistently increased the lifespan of animals. Associated with the increased lifespan is a reduced and/or delay in the onset of various age-related diseases. Unfortunately, the biological mechanism responsible for these observations remains unknown. As a first approach to this problem, studies have been carried out to relate the biochemical characteristics of the tissues of animals subjected to different dietary regimens known to increase lifespan. These results have supported a working hypothesis that increased lifespan associated with reduced dietary protein intake may be related to the rate of genetic informational transfer.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)U.S. DEPARTMENT OF  
HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00081-05-LCP

## PERIOD COVERED

July 1, 1976 to September 30, 1977

## TITLE OF PROJECT (80 characters or less)

Age Effects on Proliferation and Differentiation of Immune Cells

## NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: T. Makinodan

Chief, LCCP

LCP NIA (DOD 8/15/76)

Other: P. L. Mann

Visiting Fellow

LCP NIA

## COOPERATING UNITS (if any)

M. H. Heidrick, University of Nebraska, Omaha, Nebraska

K. Hirokawa, Tokyo Medical &amp; Dental University, Tokyo, Japan

## LAB/BRANCH

Gerontology Research Center, Laboratory of Cellular and Comparative Physiology

## INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

## TOTAL MANYEARS:

0.28

## PROFESSIONAL:

0.18

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS☐ (b) HUMAN TISSUES☒ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

## SUMMARY OF WORK (200 words or less - underline keywords)

The long term goal of this project is to understand the cellular and molecular mechanisms for loss of immunologic vigor with age and to promote immuno-restoration. Present studies are focused on: (a) cellular etiology of immunosenescence, (b) the development of simple sensitive methods to assess the immunologic potential of peripheral blood cells and (c) the development of immunorestorative methods.

## Project Description:

This project has been terminated.

## Publications:

Albright, J.W. and T. Makinodan: Decline in the growth potential of spleen colonizing bone marrow stem cells of long-lived aging mice. J. Exp. Med. 144: 1204-1213, 1976.

Hirokawa, K., Albright, J.W. and Makinodan, T.: Restoration of impaired immune functions in aging animals. I. Effect of syngeneic thymus and bone marrow grafts. Clin. Immunol. Immunopath. 5: 371-376, 1976.

Kay, M.M.B. and Makinodan, T.: Immunobiology of aging: Evaluation of current status. Clin. Immunol. Immunopath. 6: 394-413, 1976.

Editor: Makinodan, T. and Yunis, E. (Eds.): Immunology and Aging. New York, Plenum Medical Book Co., 1977, 208 pp.

Makinodan, T.: Biology of Aging: Retrospect and Prospect. In Makinodan, T. and Yunis, E. (Eds.): Immunology and Aging. New York, Plenum Medical Book Co., 1977, pp. 1-17.

Makinodan, T.: Immunity and Aging. In Finch, C.E. and Hayflick, L. (Eds.): Handbook of the Biology of Aging. New York, Van Nostrand Reinhold, 1977, pp. 379-408.

Makinodan, T.: Immunobiology of aging. J. Amer. Geriatr. Soc. 24: 249-252, 1976.

Makinodan, T., Albright, J.W., Good, P.I., Peter, C.P. and Heidrick, M.L.: Reduced humoral immune activity in long-lived old mice: an approach to elucidating its mechanism. Immunology 31: 903-911, 1976.

Makinodan, T., Good, R.A. and Kay, M.M.B.: Cellular Basis of Immunosenes-  
cence. In Makinodan, T. and Yunis, E. (Eds.): Immunology and Aging. New York, Plenum Medical Book Co., 1977, pp. 9-22.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01-AG-00085-05-LCP
PERIOD COVERED July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (80 characters or less)  Diet Probes to Study Aging Immunologic and Biochemical Functions		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <div style="display: flex; justify-content: space-between;"> <div> PI: G. H. Stoltzner Other: T. Makinodan </div> <div> Staff Fellow Chief, LCCP </div> <div> LCP NIA (DOD 5/31/76) LCP NIA (DOD 8/15/76) </div> </div>		
COOPERATING UNITS (if any)  None		
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Comparative Physiology		
SE		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  <u>Protein restriction</u> , in various degrees and duration, has resulted in <u>increased life expectancy</u> in older <u>mice</u> . This finding is being correlated with a number of assays in an attempt to understand the mechanism of this life prolongation effect.		

Project Description:

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01-AG-00086-03-LCP								
PERIOD COVERED July 1, 1976 to September 30, 1977										
TITLE OF PROJECT (80 characters or less)  The Rat as a Model for the Immunologic Study of Aging										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table border="0"> <tr> <td>PI:</td> <td>G. H. Stoltzner</td> <td>Staff Fellow</td> <td>LCP NIA (DOD 5/31/76)</td> </tr> <tr> <td>Other:</td> <td>T. Makinodan</td> <td>Chief, LCCP</td> <td>LCP NIA (DOD 8/15/76)</td> </tr> </table>			PI:	G. H. Stoltzner	Staff Fellow	LCP NIA (DOD 5/31/76)	Other:	T. Makinodan	Chief, LCCP	LCP NIA (DOD 8/15/76)
PI:	G. H. Stoltzner	Staff Fellow	LCP NIA (DOD 5/31/76)							
Other:	T. Makinodan	Chief, LCCP	LCP NIA (DOD 8/15/76)							
COOPERATING UNITS (if any)  None										
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Comparative Physiology S:										
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224										
TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0								
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords)  Utilizing the <u>rat</u> as a model of mammalian aging, quantification of <u>age-dependent decline in lymphocyte proliferative ability</u> when stimulated by lectins and allogeneic cells is being performed.										

Project Description:

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01-AG-00087-04-LCP								
PERIOD COVERED July 1, 1976 to September 30, 1977										
TITLE OF PROJECT (80 characters or less) Mechanism of the Parental Age Effects										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT										
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">E. L. Schneider</td> <td style="width: 35%;">Medical Officer, PHS</td> <td style="width: 15%;">LCP NIA</td> </tr> <tr> <td>Other:</td> <td>D. Kram</td> <td>Staff Fellow</td> <td>LCP NIA</td> </tr> </table>			PI:	E. L. Schneider	Medical Officer, PHS	LCP NIA	Other:	D. Kram	Staff Fellow	LCP NIA
PI:	E. L. Schneider	Medical Officer, PHS	LCP NIA							
Other:	D. Kram	Staff Fellow	LCP NIA							
COOPERATING UNITS (if any) Dr. Barton Gledhill, UCA Lawrence Livermore Lab, Livermore, CA Dr. Morris Pollard, D/Microbiol, Notre Dame U, South Bend, IN										
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Comparative Physiology										
SE										
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224										
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0								
CHECK APPROPRIATE BOX(ES)										
<input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER										
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords) With increasing <u>parental age</u> , there is an exponential increase in the frequency of children born with <u>chromosomal disorders</u> . Most studies indicate that this effect is chiefly due to <u>maternal aging</u> . We have developed a <u>mouse model</u> for examining this maternal age effect and have demonstrated a highly significant increase in the frequency of chromosomally abnormal mouse fetuses with maternal aging. Studies conducted on mice have indicated that neither <u>immunologic deficiency</u> nor <u>genetic predisposition</u> appear to play a strong role in the maternal age related increase in chromosomally abnormal offspring. Current research is directed at examining other proposed etiologic agents for the maternal age effect as well as analyzing the potential role of <u>paternal aging</u> .										

## Project Description:

Objectives: It has been clearly established that with increased maternal age there is a greatly increased risk of children being born with chromosomal disorders. Despite considerable speculation about the cause of this maternal age effect, research to delineate the mechanisms of this effect has been limited by the practical as well as ethical considerations of human experimentation. This problem has been approached in this laboratory by utilizing the mouse as an animal model since it has been demonstrated that with increased mouse maternal age there is an increased frequency of chromosomally abnormal fetuses. Further studies were directed at examining the genetic and immunological components of the maternal age effect. Although minor differences were observed in the age-related increase in chromosomally abnormal offspring between various mouse inbred strains, no substantial genetic component could be defined. Immune deficiency did not appear to significantly affect the frequency of chromosomally abnormal fetuses.

Recent evidence indicates that advanced paternal age may also contribute to the increased frequency of chromosomally abnormal offspring with advanced parental age. This paternal age component can be directly evaluated by examining the chromosomal complement of human sperm samples derived from volunteer members of the Baltimore Longitudinal Study.

### Methods Employed:

1. Sperm samples obtained from young and old volunteer members of the Baltimore Longitudinal Study were sent to Dr. Barton Gledhill of the University of California Lawrence Livermore Laboratories for flow microfluorometric analysis. With flow microfluorometric analysis, the DNA contents of individual sperm can be measured with great accuracy.
2. Sperm samples from Longitudinal subjects were also placed on slides and stained with the appropriate fluorescent stains to detect fluorescence of the Y chromosome. These Y bodies are an indicator of the number of Y chromosomes present in a single sperm. The number of double Y bodies indicating the presence of two Y chromosomes would reflect the overall incidence of chromosomal aneuploidy.

### Major Findings:

1. Manuscripts have been prepared detailing our early findings of a lack of a strong genetic or immunological component to the maternal age effect.
2. Preliminary results of flow microfluorometric analysis indicated that this technique can be applied to examining chromosomal aneuploidy in human sperm samples. Results obtained by flow microfluorometry appear to correlate well with staining for Y bodies.



### Significance to Biomedical Research and the Program of the Institute:

Chromosomal disorders are extraordinarily common in man with a frequency of approximately 1 in 100 live births. This frequency is considerably higher if one considers that over one-half the spontaneous abortions that occur during pregnancy are due to chromosomal abnormalities. With increasing maternal age, the risk of having a child with a chromosomal abnormality, such as Down's syndrome (mongolism), increases dramatically. A mother at age 45 or above has a 100-fold greater chance of having a child with this syndrome than a mother aged 15 to 20. It is, therefore, of great clinical importance that insight into the mechanisms of this maternal age effect be obtained.

The mouse has proved to be an appropriate animal model for studying this maternal age effect since a marked increase in the frequency of chromosomally abnormal embryos has been observed with increasing maternal age. A survey of mouse inbred strains did not reveal significant differences in the maternal age effect between strains but instead indicated that the maternal age effect was present to a similar degree in all strains examined. These results suggest a lack of a strong genetic component of the maternal age effect. Similarly, comparison of the frequency of chromosomally abnormal embryos between mice in which immune incompetence was induced and controls did not reveal a significant increase in aneuploidy as a function of altered immunity. Therefore, if the results of these studies can be applied to man, they would suggest that neither genetic predisposition nor altered immunity play a vital role in the increased frequency of chromosomally abnormal offspring born to older mothers.

Proposed Course: Studies of the maternal age effect will be continued by examining other proposed etiologic agents. The possible role of infectious agents will be assessed by analyzing the frequency of chromosomally abnormal fetuses in germ-free mice in collaboration with Dr. Morris Pollard.

Studies of the paternal age effect will continue with flow microfluorometric and cytologic analysis of sperm samples obtained from volunteer members of the Baltimore Longitudinal Study. In addition, an attempt will be made to find a mouse model for the paternal age effects seen in humans.

### Publications:

Schneider, E.L.: The Dilemma of Aging Parents: Increased Risk of Genetically Abnormal Offspring. In Behnke, J., Finch, C. and Moment, G. (Eds.): Biology of Aging, in press.

Kram, D. and Schneider, E.L.: An Effect of Reproductive Aging: Increased Risk for Genetically Abnormal Offspring. In Schneider, E.L. (Ed.): The Aging Reproductive Systems. New York, Raven Press, in press.

Kram, D. and Schneider, E.L.: Parental Age Effects. In Schneider, E.L. (Ed.): The Genetics of Aging. New York, Plenum Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00088-05-LCP
PERIOD COVERED July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (80 characters or less) Mechanisms of Cellular Aging		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	E. L. Schneider D. Kram Y. Nakanishi G. Bynum P. Thorne	Medical Officer, PHS Staff Fellow Visiting Fellow Medical Officer, PHS Chief, Tech Dev Sec  LCP NIA LCP NIA LCP NIA(EOD 1/7/77) LCP NIA(EOD 7/18/77) OC NIA
COOPERATING UNITS (if any) W. Adler, LCP NIA J. Smith, W. Alton Jones Cell Science Center, Lake Placid, NY G. Sack, Johns Hopkins School of Medicine, Baltimore, MD P. Huang, Johns Hopkins School of Public Health & Hygiene, Balto, MD		
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Comparative Physiology		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS: 3.75	PROFESSIONAL: 2.75	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Our major objectives are to examine <u>cell replication</u> and <u>repair of DNA damage</u> as a function of <u>aging in human cells in vitro</u> and in <u>rat and mouse tissues in vivo</u> . We have continued our studies of skin fibroblast cultures derived from human volunteer subjects and found additional evidence for a loss of replicative ability with <u>in vivo</u> aging. A technique has been developed to examine cell replication <u>in vivo</u> in intact animals utilizing the BrdU-differential staining technique. Preliminary results obtained with this technique indicate a decline in cell replication as a function of aging. Further research will be directed not only at defining the decline in cell replication but also at investigating the mechanisms for this functional loss. The BrdU-differential staining techniques have also been applied to examining DNA repair by analyzing the frequencies of sister chromatid exchanges (SCE) induced by various DNA damaging agents (i.e., mitomycin C) (MMC) <u>in vivo</u> in mouse and rat bone marrow cells and <u>in vitro</u> in cultured human fibroblasts. In all these systems, a significant decrease in MMC-induced SCE was observed in the older cell populations. The mechanism for the altered response to DNA damage as manifested by diminished SCE will be investigated.		

## Project Description:

**Objectives:** A decline in the proliferative capacities of certain cell populations is an important feature of human aging. Because of the practical and ethical limitations of in vivo human experimentation, considerable effort has been expended to create appropriate in vitro tissue culture systems as well as in vivo animal models for examining age-related alterations in cell replication.

There has been considerable concern expressed over whether in vitro human diploid fibroblast aging reflects in vivo human aging. To examine this important question, cell replicative capacity was measured in a series of human fibroblast cultures derived from old and young healthy volunteer members of the Baltimore Longitudinal Study. In this manner, many of the in vitro aging parameters could be analyzed as a function of in vivo age.

Although in vitro studies of cell replication are important, they are like all in vitro work restricted by the limitations of an artificial environment. It was, therefore, vital that an in vivo technique be developed to examine cell replicative abilities as a function of age. Previous in vivo studies were performed with radioactive labeling or by examining the end result of cellular proliferation. However, the advent of new cytogenetic techniques allows for direct examination of cell replication as a function of age without the need for radioisotopes. With these techniques, cell replication will be studied in several animal systems.

Another aspect of this project will be to examine the mechanisms for the age-associated decline in cell replication. One important function that is crucial to DNA replication is the ability of replicating cells to repair DNA damage. It was, therefore, decided to investigate the ability of replicating cells to repair DNA damage both in vitro and in vivo as a function of age.

**Methods Employed:**

1. The replicative capacities of skin fibroblast cultures derived from old and young normal volunteer members of the Baltimore Longitudinal Study were assessed by measuring the following parameters: outgrowth of cells from the biopsy tissue, cell population doubling time, cell density at confluency, cumulative cell population doublings, and colony size distributions.
2. For measurement of cell replication in vivo, young and old animals were infused intravenously with BrdU. At increasing time intervals, these animals were sacrificed and chromosomal preparations were made. Cells that had undergone 1, 2 and 3 cell cycles in the presence of this drug could be clearly identified by characteristic banding patterns.
3. By utilizing the BrdU-differential staining technique both in vitro and in vivo, one can measure the frequency of sister chromatid exchanges (SCE). This frequency reflects the ability of cells to repair DNA damage.

Major Findings:

1. Skin fibroblast cultures derived from old (ages 65-95) human donors were found to have diminished replicative capabilities when compared to parallel cultures derived from young donors (ages 20-35). Statistically significant differences in cell outgrowth rate, cell population doubling time, cell density at confluency, cumulative cell population doublings, and colony sizes were observed. Of particular interest were the colony size distributions which appear to be an accurate indicator of both in vivo and in vitro aging.
2. Utilizing the BrdU-differential staining techniques, we have developed a new approach to measuring cell replication in vivo as well as in vitro. The technique is rapid, reproducible, capable of determining cell cycle times and can be performed under conditions where cellular replication can be demonstrated to not be affected by the procedure.
3. With this new technique, preliminary studies of human lymphocyte cultures in vitro and rat bone marrow cells in vivo have revealed a clear decrease in cell replication as a function of aging.
4. Analyses of SCE frequencies have demonstrated a high statistically significant decline in these events in vitro in human skin fibroblast cultures derived from old donors (compared with cells from young donors) and in late passage IMR-90 cells (when compared with early passage cells, "in vitro aging"). Examination of bone marrow cells from C57BL/6 mice and Wistar rats, where studies were conducted entirely in vivo, also revealed a significant decline in the frequency of mitomycin C (MMC) induced SCEs. To demonstrate that these findings were not due to a specific response to MMC, similar results were obtained in vitro with EMS and AAAF, two potent alkylating agents, and in vivo with cyclophosphamide.
5. The frequencies of chromosomal aberrations (CA) in response to MMC were also increased in old cells when compared with young cells both in vivo and in vitro.
6. Examination of MMC and cyclophosphamide induced SCE in AKR mice, which display a predisposition toward malignancy and early mortality, revealed a similar decline in SCE frequencies.
7. Measurement of SCE frequencies in vivo as a function of BrdU dosage revealed that below certain concentrations the frequency of SCE remained stable at 1.5. This strongly supports the spontaneous nature of SCE.

Significance to Biomedical Research and the Program of the Institute.

Analysis of skin fibroblast cultures derived from young and old human volunteers reveals a decrease in replicative potentials as a function of the age of the cell donor. These results not only support the use of cell cultures to study human aging but also introduce an alternate cell model to early and late passage human fetal lung cell cultures (WI-38) for the study of cellular aging.

The application of the BrdU-differential staining technique to cell kinetic measurements has provided a new and sensitive tool to examine cell proliferation both in vitro in cultured human cells and in vivo in intact animals. Preliminary studies have already shed light on the controversy over whether cell proliferation is diminished with in vivo aging. These studies indicate a consistent decline in cell replication both in vivo and in vitro.

Measurements of SCE frequencies were shown to be a sensitive indicator of DNA damage both in vivo and in vitro. Our studies in four separate systems all indicate an altered response of old cells to induced DNA damage. This, together with the observed increased frequencies of CA, would suggest a significant alteration in DNA repair capabilities with cellular aging.

The finding of impaired SCE formation in AKR mice at an early age indicates that alterations in DNA repair by the mechanism of SCE may play a role in the development of malignancy which characterizes this mouse strain and leads to its early death.

Proposed Course: This past year has been focused on the development of the technology for measuring cellular replication and DNA repair in vivo as well as in vitro. Both processes appear to be significantly altered with aging. These techniques, therefore, will now be aimed at elucidating the mechanisms for the decline in these vital cellular capabilities.

Studies of the AKR mouse strain will continue, since this strain may provide a genetic mutant which features premature aging for the DNA repair pathway involving SCE.

Studies of skin fibroblast cultures derived from old and young human volunteer members of the Baltimore Longitudinal Study will continue with an emphasis on collaboration with outside laboratories with expertise in a variety of areas ranging from transformability of these cells with SV<sub>40</sub> virus, cell mobility, antioxidant properties, and cyclic nucleotide metabolism.

#### Publications:

Schneider, E.L. and Fowlkes, B.J.: Measurement of DNA content and cell volume in senescent human fibroblasts utilizing flow multiparameter single cell analysis. Exp. Cell Res. 98: 298-303, 1976.

Mitsui, Y. and Schneider, E.L.: Relationship between cell replication and volume in senescent human diploid fibroblasts. Mech. Ageing Develop. 5: 45-56, 1976.

Mitsui, Y. and Schneider, E.L.: Increased nuclear sizes in senescent human diploid fibroblast cultures. Exp. Cell Res. 100: 147-152, 1976.

Schneider, E.L., Chaillet, J. and Tice, R.: In vivo BrdU labeling of mammalian chromosomes. Exp. Cell Res. 100: 396-399, 1976.

Schneider, E.L. and Mitsui, Y.: Examination of the relationship between in vitro cellular aging and in vivo human age. Proc. Natl. Acad. Sci. (USA) 73: 3584-3588, 1976.

Tice, R., Schneider, E.L. and Rary, J.M.: The utilization of bromodeoxy-uridine incorporation into DNA for the analysis of cellular kinetics. Exp. Cell Res. 102: 232-236, 1976.

Tice, R., Chaillet, J. and Schneider, E.L.: Demonstration of spontaneous sister chromatid exchanges in vivo. Exp. Cell Res. 102: 426-428, 1976.

Mitsui, Y. and Schneider, E.L.: Characterization of fractionated human diploid fibroblast cell populations. Exp. Cell Res. 103: 23-30, 1976.

Schneider, E.L., Sternberg, H. and Tice, R.: In vivo analysis of cellular replication. Proc. Natl. Acad. Sci. (USA) 74: 2041-2044, 1977.

Schneider, E.L., Mitsui, Y., Au, K.S. and Shorr, S.S.: Tissue-specific differences in cultured human diploid fibroblasts. Exp. Cell Res., in press.

Schneider, E.L.: In Vivo vs In Vitro Human Cellular Aging. In Harrison, D. (Ed.): Genetic Effects on Aging. National Foundation Series, in press.

Schneider, E.L.: Cytogenetics of Aging. In Schneider, E.L. (Ed.): The Genetics of Aging. New York, Plenum Press, in press.

Schneider, E.L., Tice, R.R. and Kram, D.: Bromodeoxyuridine-differential Staining Technique. A New Approach to Examining Sister Chromatid Exchange and Cell Replication Kinetics. In Prescott, D. (Ed.): Methods in Cell Biology. New York, Academic Press, in press.

Schneider, E.L. and Kram, D.: Sister Chromatid Exchanges and Aging. In Nichols, W. (Ed.): Proceedings of Camden Workshop on DNA Repair and Aging. New York, Plenum Press, in press.

Stanbridge, E.J. and Schneider, E.L.: A simple biochemical method for the detection of mycoplasma and other microbial contaminants of cell cultures. Tissue Culture Assoc. Manual 2: 371-374; 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00089-03-LCP								
PERIOD COVERED July 1, 1976 to September 30, 1977										
TITLE OF PROJECT (80 characters or less) Human Immunology Program										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT										
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 30%;">M. M. B. Kay</td> <td style="width: 30%;">Medical Officer, PHS</td> <td style="width: 25%;">LCP NIA (DOD 1/31/77)</td> </tr> <tr> <td>Other:</td> <td>T. Makinodan</td> <td>Chief, LCCP</td> <td>LCP NIA (DOD 8/15/76)</td> </tr> </table>			PI:	M. M. B. Kay	Medical Officer, PHS	LCP NIA (DOD 1/31/77)	Other:	T. Makinodan	Chief, LCCP	LCP NIA (DOD 8/15/76)
PI:	M. M. B. Kay	Medical Officer, PHS	LCP NIA (DOD 1/31/77)							
Other:	T. Makinodan	Chief, LCCP	LCP NIA (DOD 8/15/76)							
COOPERATING UNITS (if any) S. Sterioff, Renal Transplantation Service, Balto. City Hosps., Baltimore, MD H. Fudenberg, Dept. of Basic & Clinical Immunology & Microbiology, Med. Univ. of S. Carolina, Charleston, S.C.										
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Comparative Physiology										
SECTION										
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224										
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 33%;">OTHER:</td> </tr> <tr> <td>1.02</td> <td>0.12</td> <td>0.9</td> </tr> </table>			TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	1.02	0.12	0.9		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:								
1.02	0.12	0.9								
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to determine which <u>immune indices</u> , measured predominately by <u>in vitro</u> assays, <u>correlate best with in situ immune function</u> and to miniaturize these assays. The topics presented include (1) optimal <u>culture conditions for PHA response</u> , (2) comparative studies on <u>human spleen</u> , <u>lymph nodes</u> , and <u>peripheral blood</u> , (3) comparative studies between immunologic parameters of human and mouse lymph nodes and spleen, and (4) studies on peripheral blood and splenic cells from patients with <u>hairy cell leukemia</u> .										

## Project Description:

This project has been terminated.

## Publications:

Kay, M.M.B.: Autoimmune disease: the consequence of deficient T cell function? J. Amer. Geriat. Soc. 24: 253-257, 1976.

Kay, M.M.B.: Hodgkin's Disease: A War between T Lymphocytes and Transformed Macrophages? In Mathe, G., Florentin, I. and Simmler, M.-C. (Eds.): Recent Results in Cancer Research. New York, Springer-Verlag, 1976, Vol. 56, pp. 111-121.

Kay, M.M.B.: High Resolution Scanning Electron Microscopy and its Application to Research on Immunity and Aging. In Makinodan, T. and Yunis, E. (Eds.): Immunology and Aging. New York, Plenum Medical Book Co., 1977, pp. 135-150.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF</b> INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01-AG-00091-03-LCP								
PERIOD COVERED July 1, 1976 to September 30, 1977										
TITLE OF PROJECT (80 characters or less)  Mechanism of Removal of Senescent Cells by Human Macrophages <u>In Situ</u>										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 30%;">M. M. B. Kay</td> <td style="width: 30%;">Medical Officer</td> <td style="width: 25%;">LCP NIA (DOD 1/31/77)</td> </tr> <tr> <td>Other:</td> <td>S. J. Hausman</td> <td>Staff Fellow</td> <td>LCP NIA</td> </tr> </table>			PI:	M. M. B. Kay	Medical Officer	LCP NIA (DOD 1/31/77)	Other:	S. J. Hausman	Staff Fellow	LCP NIA
PI:	M. M. B. Kay	Medical Officer	LCP NIA (DOD 1/31/77)							
Other:	S. J. Hausman	Staff Fellow	LCP NIA							
COOPERATING UNITS (if any)  None										
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Comparative Physiology										
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224										
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:								
0.9	0.8	0.1								
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to determine the mechanism by which macrophages distinguish between adult and <u>senescent self</u> . Experiments indicate that human macrophages make this distinction on the basis of <u>selective IgG attachment</u> to the surface of senescent cells. The objective of this project is to isolate and characterize the <u>regulatory IgG</u> . The antibody eluted from senescent cells was shown to be (1) an IgG without other immunoglobulins by immunodiffusion, immunoelectrophoresis, and acrylamide gel electrophoresis, (2) polyclonal, and (3) a <u>regulatory autoantibody</u> .										

## Project Description:

This project has been terminated.

## Publications:

Kay, M.M.B.: Aging and the Decline of Immune Responsiveness. In Fudenberg, H.H., Sites, D.P., Caldwell, J.L. and Wells, J.V. (Eds.): Basic and Clinical Immunology. Los Altos, Lange Medical Publishers, 1976, pp. 267-268.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  201-AG-00093-05-LCP
PERIOD COVERED July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (80 characters or less)  Cellular Basis of Regulation of the Humoral Immune Response		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:  Other:	A. A. Nordin  P. L. Mann	Research Chemist  Visiting Fellow  LCP NIA  LCP NIA
COOPERATING UNITS (if any)  None		
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Comparative Physiology		
SL		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS:  1.6	PROFESSIONAL:  0.8	OTHER:  0.8
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  The regulatory role of lymphoid cells and their products on the humoral immune response is the intent of this project. The <u>regulatory mechanisms</u> are being investigated <u>in vitro</u> using a T-cell dependent as well as a T-cell independent antigen. The cellular requirements and functions involved in the <u>in vitro immune response</u> are being established for normal adult mice. These are then being compared to those in aged mice of the same strains. The possibility that regulatory mechanisms observed in young adult mice are amplified in old mice resulting in <u>immunosenescence</u> is being investigated.		

## Project Description:

Objectives: The goal of this project is to characterize the cells regulating the immune response by cellular elements in both young and aged mice. Efforts to determine the origin and mechanism of action of these cells are of prime interest.

Methods Employed: (1) The in vitro culture techniques and the assay for plaque-forming cells are routine methods.

(2) Carbonyl-iron treated spleen cells are performed by adding 25 mg of sterile carbonyl iron to  $100 \times 10^6$  normal spleen cells. After a 30 minute incubation at 37°C in a 5% CO<sub>2</sub> environment, the iron and cells with ingested iron are removed by magnetic attraction. This process is repeated and the spleen cells free of iron-ingesting cells are used as a source of T and B lymphocytes.

(3) Peritoneal exudate cells are collected from unstimulated mice and used as a source of accessory cells. These cells are either used directly or an adherent layer prepared from them before other cell types are added. Supernatants of peritoneal cells collected 24 hours after culture initiation are used in some instances instead of the peritoneal cells.

(4) DAGG-Ficoll is prepared by modifying Ficoll by introducing carboxyl methyl amino-ethyl groups to which is added the tri-peptide glycine-glycyl-alanyl with the terminal alanine substituted with a single dinitro-phenol haptenic group. The preparation used here contains 48 moles of hapten per mole of Ficoll.

Major Findings: The in vitro humoral immune response to the T independent antigen, DAGG-Ficoll, is completely dependent on the presence of macrophages. There is an optimum number of macrophages required but the magnitude of the response can be significantly enhanced if 2-mercapto-ethanol is also added. The mercaptoethanol effect however is not mediated through a macrophage system but rather seems to be functioning as a non-specific activator of lymphocytes.

The supernatant fluids of macrophage culture for 24 hours can substitute for this cell function but only in the presence of mercaptoethanol. The factor elaborated by macrophages is independent of the presence of antigen or ME. Although the macrophage factor does have a polyclonal activator effect, the vast majority of the effect of the factor is exerted on specific antibody synthesis. The factor can be derived from allogeneic macrophages and is not dependent on the presence of T cells for synthesis or release. The factor is resistant to mild heating and remains active when stored at -70°C.

Peritoneal cells cultured for 24 hours and washed are no longer able to support the in vitro immune response. On the other hand, lymphocytes cultured for 24 hours retain the majority of their activity.

Significance to Biomedical Research and the Program of the Institute: The goal of this research program is to examine the cellular populations that are regulating the humoral immune response. The mechanisms by which the regulation takes place would be of significance not only to the field of immunology but would have relevance to cell biology. It is also significant to the area of immunosenescence. The decline in immunological responsiveness with age is well established but the reasons are not at all understood. The role of regulatory mechanisms in explaining the phenomena of immunosenescence may be of considerable significance.

Proposed Course: The previously defined deficiency of old mouse spleen cells to respond in vitro will be examined to determine if this deficiency is due to a lack of macrophage function. Also the characterization of the effect of the macrophage function will be continued in an effort to further define the nature and mechanism of action of the factor(s).

Publications:

Schreier, M.H. and Nordin, A.A.: An Evaluation of the Immune Response In Vitro. In Loor, F. and Roelants, G.E. (Eds.): B and T Cells in Immune Response. New York, John Wiley and Sons, 1977, pp. 127-151.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01-AG-00094-04-LCP
PERIOD COVERED July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (80 characters or less) Characterization of Immune System of Aging Mice with Immunodeficiency		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <div style="display: flex; justify-content: space-between;"> <span>PI: A. A. Nordin</span> <span>Research Chemist</span> <span>LCP NIA</span> </div>		
COOPERATING UNITS (if any)  None		
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Comparative Physiology		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 0.5	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  The purpose of this project is to characterize the <u>in vitro</u> cell-mediated <u>immunity</u> in young and aging mice of various genetic strains. The relationship between the <u>immunodeficiency in aging</u> and the regulation, in the form of suppression of the immune system, is investigated. The topics of present interest are: (1) the regulatory mechanisms of the <u>in vitro</u> development of <u>cytotoxic lymphocytes</u> by <u>suppressor cells</u> , (2) the characterization of suppressor cells, and (3) the characterization of the immunodeficiency in aging and its relationship with suppressor cells.		

## Project Description:

**Objectives:** The goal of this project is to characterize the immune system in young and aging mice of various genetic strains. The relationship between the immunological disorders and the regulation, in the form of suppression of the immune system, is investigated.

**Methods Employed:** (1) A modification of the spleen cell culture system of Mishell and Dutton is used. Spleen cells from individual mice or pooled spleen cells are cultured with mitomycin-C treated or irradiated allogeneic spleen cells,  $F_1$  spleen cells or heterologous erythrocytes at 37°C for various days. The double chamber culture system is also used.

(2) Cytotoxicity assay -  $^{51}\text{Cr}$  labelled EL-4 or P-815 cells are mixed with cultured spleen cells and incubated for various times. After incubation, cold PBS is added, the tubes centrifuged and the radioactivity of the supernatant counted.

(3) Plaque-forming cell assay-routine technique used to detect IgM and IgG antibody-producing cells.

**Major Findings:** The generation of cytotoxic lymphocytes in vitro was further studied in young and old C57Bl/6 mice to determine the mechanism(s) of the previously defined suppressive effects. An attempt was made to determine if the suppression of cytotoxic lymphocyte development by a sub-population of T-cells was expressed by a soluble factor(s) elaborated by such cells. Supernates from 2 day microcultures of mixed lymphocyte cultures were shown to significantly suppress the development of cytotoxic lymphocytes in vitro. The factor(s) is resistant to mild heat treatment and is not retained by a 300,000 MW exclusion filter. Attempts to isolate the factor(s) by column chromatography have not been successful to date.

Old C57Bl/6 mice were examined on an individual basis to correlate the in vitro development of cytotoxic lymphocytes with the production of suppressor cells and factors. Spleen cells taken directly from old mice are not suppressive. Old spleen cells in mixed lymphocyte cultures do develop suppressor cells and, as expected, the supernate is also active.

**Significance to Biomedical Research and the Program of the Institute:** This proposal offers two main significant contributions: (1) the regulatory mechanism of cell-mediated immunity in young and aged mice, and (2) the mechanism of the immunosenescence of cell-mediated immunity.

**Proposed Course:** The isolation and further characterization of the suppressive factor(s) will be attempted. Also the spleen cells of old mice will be studied to determine if the lack of the in vitro development of cytotoxic lymphocytes is related to the amount of suppressive factor elaborated. Attempts will be made to demonstrate the development and role of suppressor cells in vivo.

## Publications:

Hirano, T. and Nordin, A.A.: Age-associated decline in the in vitro development of cytotoxic lymphocytes in NZB mice. J. Immunol. 117: 1093-1098, 1976.

Hirano, T. and Nordin, A.A.: Cell-mediated immune response in vitro. II. The mechanisms involved in the suppression of the development of cytotoxic lymphocytes. J. Immunol. 117: 2226-2232, 1976.



## PERIOD COVERED

July 1, 1976 to September 30, 1977

## TITLE OF PROJECT (80 characters or less)

The Role of Cell Membrane Structures on Cellular Recognition

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER  
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. H. Adler	Medical Officer, PHS	LCP NIA
Other:	J. E. Nagel	Clinical Associate, PHS	LCP NIA (EOD 7/1/77)
	K. H. Jones	Staff Fellow	LCP NIA
	S. J. Hausman	Staff Fellow	LCP NIA
	M. A. Brock	Research Biologist	LCP NIA
	H. Nariuchi	Visiting Fellow	LCP NIA (DOD 8/25/76)
	J. W. Heine	Staff Fellow	LCP NIA (DOD 8/27/76)

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Gerontology Research Center, Laboratory of Cellular  
and Comparative Physiology

SU

## INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

## TOTAL MANYEARS:

3.0

## PROFESSIONAL:

2.3

## OTHER:

0.7

## CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS☐ (b) HUMAN TISSUES☒ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

## SUMMARY OF WORK (200 words or less - underline keywords)

Lymphoid cells from the spleens of old mice demonstrate poor cell division in several mitogen assay systems. The poor response can be explained by a decrease in the number of responsive cells, defective daughter cells which will not divide, and the presence of inhibitors which interfere with division. All these factors can be seen as causes of decreased cellular function in old mice. Furthermore, the immune memory of T cells in mice is relatively short-lived so that secondary responses are lost in a year's time or less. Loss of memory, plus a decrease in responsiveness of T cells seen with age may explain the age-related defect in cell-mediated immunity.

Identification of cell types has been facilitated by the development of cellular adaptation culture techniques and newer techniques for labeling antibody protein with fluorescent dyes.

Another phase of this project has been the study of the anamnestic response of cytotoxic T cells. Mice were immunized against a tumor allograft and then tested over the following 12 months for their ability to show a secondary cell-mediated cytotoxic response. A strong secondary response was measured over the first 3 months after allograft. In fact, after 3 months, only 1/50th the amount of antigen was needed to stimulate a secondary response as was needed to initiate a primary response in age-matched nonsensitized controls. The intensity of the secondary response continued to decrease, showing considerable variation among individuals from 4 to 6 months, and was finally lost 12 months after immunization. Cell-mediated immunity is exceedingly sensitive to age-related impairment and these data show that immunization procedures engender relatively short-lived memory in the system. This may help to explain why the age-related impairment in the function of the thymic-dependent system is so dramatic; i.e., memory falls off rapidly and the responsiveness of the system to new challenges drops with age.

Other findings related to functional assay and cellular identification procedures have been the development of adaptational culture techniques and techniques for labeling antibodies with dyes. The culture techniques have allowed us to adapt immunologically functional tumor cell lines to a totally in vitro culture system. This allows a better look at the individual cell's function without contamination by host cells which can invade the tumor in vivo. The newer procedures for the attachment of fluorescein to antibody protein has resulted in a better controlled reaction which has yielded protein with a precise, preselected amount of dye attached. This has resulted in a better battery of antisera which can be used for identification of cells in lymphoreticular tissue.

Significance to Biomedical Research and the Program of the Institute: We are gaining a better definition and appreciation of the term immunodeficiency. Since a relative immunodeficiency is seen in aging, it is important to develop better diagnostic criteria, so that possible remedial measures can be undertaken.

Proposed Course: To continue to outline the connection between form and function and to expand our technical ability to measure functions. To develop better diagnostic criteria and tests to describe immune capacity.

#### Publications:

Heine, J.W. and Adler, W.H.: The kinetics of interferon production by mouse lymphocytes and its modulating effect on the virus plaque forming cell assay as a quantitative method to determine activated lymphocytes. J. Immunol. 117: 1045-1048, 1976.

Heine, J.W. and Adler, W.H.: The quantitative production of interferon by mitogen stimulated mouse lymphocytes as a function of age and its effect on the lymphocytes proliferative responses. J. Immunol. 118: 1366-1369, 1977.

## Project Description:

Objectives: To correlate certain immunologic functions with morphologically identifiable populations of immunologically active cells. The functional criteria and results will be compared to arrive at correlations in order to arrive at methods for diagnosing and describing immune deficiency and assigning certain predictive projections of immune function. The tests of immune functions will include responses to mitogen and antigen in in vitro culture conditions, responses to antigens and oncogenic stimuli in vivo, and the development of immunologically competent cells in in vitro environments. These studies will give a better understanding of age-related immunodeficiency.

Methods Employed: The basis of most functional assays will be in vitro culture systems. There will be both short term for the investigation of mitogen and antigen responsiveness and for the generation of antibody-forming cells, and longer time for the generation of cytotoxic lymphocytes and antibody-forming cell colonies. In vivo methods will primarily be cell transfer studies and transplantation studies with syngeneic tumor cells. The cells will be from various lymphoid organs and from varying aged donors. The cells will be treated by physical separation methods and with specific antisera to eliminate certain populations, or to quantitate various cellular population representation.

Major Findings: By stimulating mouse spleen cells with a variety of mitogenic agents, it is possible to force the development of a population of cells which are daughters of the original responder cells. These daughter cells then can be subjected to another stimulating mitogen either the original or another mitogen of the same type and the response can be measured. It was found that spleen cells from young mice will respond to mitogens to develop daughters which will again respond nicely to a mitogen. The division potential of these cells is adequate for performance in several subsequent cultures. However, cells from old mice show a marked inability to respond even to a  $2^0$  stimulus and, therefore, have a markedly limited division potential. Along with our other findings that there are decreased numbers of responding units in the spleens of the old mice and there are inhibitory factors elaborated by the cells from these spleens, we are now able to appreciate the range of defects which contribute to the age-related decline in immune function.

The basic method for culturing lymphoid cells has been further refined to show that the system discriminated against both high and low responses of cells and a thymidine incorporation assay does not reflect the number of cells which are responding to a stimulus. Cells which respond poorly in culture have a disproportionately lower response than they should. Cells which respond very well can easily exhaust media resources and show a "blunted"-lower response than they should.

The cellular response to any mitogenic stimulus is dependent on the culture conditions and, therefore, it has been necessary to evaluate many of the tests we now use for assaying immune function.

Adler, W.H., Jones, K.H. and Brock, M.A.: Aging and Immune Function. In Behnke, J., Finch, C. and Moment, G. (Eds.): Biology of Aging, in press.

Adler, W.H., Jones, K.H. and Nariuchi, H.: Aging and Immune Function. In Thompson, R. (Ed): Recent Advances in Clinical Immunology. London, Churchill-Livingstone, in press.

Hausman, S.J.: Adaptation of myelomas to in vitro growth. Tissue Culture Association Manual, in press.

Ozato, K., Ebert, J.D. and Adler, W.H.: The differentiation of suppressor populations as revealed by studies of the effects of mitogens on the mixed lymphocyte reaction and on the generation of cytotoxic lymphocytes. Cell. Immunol. 22: 323-333, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF          INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER  Z01-AG-00096-04-LCP						
PERIOD COVERED July 1, 1976 to September 30, 1977								
TITLE OF PROJECT (80 characters or less) Low Temperature Effects on Cells of Aging Individuals								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: M. A. Brock</td> <td style="width: 33%;">Research Biologist</td> <td style="width: 33%;">LCP NIA</td> </tr> <tr> <td>Other: W. H. Adler</td> <td>Medical Officer, PHS</td> <td>LCP NIA</td> </tr> </table>			PI: M. A. Brock	Research Biologist	LCP NIA	Other: W. H. Adler	Medical Officer, PHS	LCP NIA
PI: M. A. Brock	Research Biologist	LCP NIA						
Other: W. H. Adler	Medical Officer, PHS	LCP NIA						
COOPERATING UNITS (if any) None								
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Comparative Physiology								
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224								
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.9	OTHER: 0						
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords) The objectives of this study are to characterize possible <u>age-related</u> <u>differences in the in vitro responses of murine lymphohemopoietic cells to</u> <u>mitogens and their differential susceptibility to freezing damage.</u> A proto- type controlled rate cooling system is currently being tested on lymphocytes of young mice. A higher than expected recovery of functional cells was ob- served at several cooling rates, which may be related to the cooling and freezing program employed. The system relies on the cell suspension temper- ature that is sensed to drive the injection of liquid N <sub>2</sub> into the cooling chamber. This method is not available in present freezing systems. A <u>cir-</u> <u>cannual rhythm of lymphocyte responsiveness to mitogens in vitro</u> was observed in a third year. Superimposed upon this rhythm were <u>seasonal changes</u> in the dose-response curve of murine lymphocytes to Concanavalin A.								

## Project Description:

Objectives: To characterize the functional capacity and structure of pre- and post-mitotic cell types from aging individuals, specifically the possible age-related differences in (1) the in vitro responses of human and murine lymphohemopoietic cells to various agents and (2) the differential susceptibility of lymphohemopoietic cells to freezing damage assessed by their functional recovery after thawing.

Methods Employed: The incorporation of  $H^3$ -thymidine into lymphocytes from mouse splenic cell suspensions was used as an indication of DNA synthesis and cell replication in vitro. The T-cell mitogens, PHA and Concanavalin A, the B-cell mitogen, LPS, several types of substrates and alcohols were added with  $H^3$ -thymidine to different experimental cultures. Murine lymphocytes in suspension with 10% DMSO and 10% fetal calf serum were frozen at rates ranging from -0.5 to -10.0°C/min in initial studies to test the prototype of the freezing system described in the next section. Recovery and viability of the cells were assessed in vitro using the mitogens listed above.

Major Findings: The lymphocytic responses to three different mitogens added to in vitro systems at increasing concentrations were followed throughout the year. In the third winter season, a decline in responsiveness to T-cell mitogens was observed. Since environmental conditions were constant, this change in murine lymphocyte responsiveness is endogenous and circannual. The dose-response curve of Concanavalin A changed seasonally. During two phases of the circannual cycle, low concentrations of Concanavalin A became increasingly less effective in stimulating DNA synthesis. An abrupt reversal to "flat" dose-response curve occurred several weeks later. The implications are not understood.

A prototype system to control the rate of cooling and the freezing of cells was tested. Other commercially available systems anticipate the point of freezing and supercool the cells before that temperature is reached. In contrast, our system senses the temperature of the cell suspension and uses that to drive the cooling system. Cells effectively control both the rate of cooling and the timing of the rapid introduction of liquid  $N_2$  at precisely the time that the heat of fusion is released. A programmable microprocessor controls the cooling and compensates for the changing characteristics of water at different low temperatures, to -60°C. We observed that this method of freezing results in high lymphocyte viability, especially that of the B cells, at all freezing rates tested. It may be that the "optimal freezing rate" described for each different cell type using conventional systems depends more on the large amplitude changes in the temperature of the cell suspensions at the point of freezing than on the rate alone. This possibility will be further tested.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF</b> <b>INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER  Z01-AG-00098-03-LCP
PERIOD COVERED July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (80 characters or less) Characterization of Alterations in Lymphocyte Membrane Structural Components		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	J. W. Heine W. H. Adler	Staff Fellow Medical Officer, PHS  LCP NIA (DOD 8/27/76) LCP NIA
COOPERATING UNITS (if any) None		
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Comparative Physiology		
SI		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The numbers of <u>lymphocytes</u> activated early in the <u>response</u> to a <u>mitogen</u> could be determined by a <u>viral plaque assay</u> . This assay showed that there were <u>less cells</u> in the spleens of the <u>old mice</u> which were able to be stimulated by the mitogen. There appeared to be the <u>lack</u> of a <u>cell population</u> in the <u>old spleens</u> which were the earliest group seen to be responsive in the young spleens. It was also found that the <u>spleen cells</u> from the <u>old mice</u> <u>produced</u> 10 times the amount of <u>interferon</u> as did cells from young mice. The <u>interferon</u> was shown to be very <u>suppressive</u> on the <u>proliferative response</u> of normal cells from young mice. The <u>defect</u> in cellular proliferation in the cells from spleens of of mice could be both a <u>lack of responsive cells</u> and the <u>presence</u> of <u>inhibitory substances</u> in the cultures of old cells.		



**Project Description:**

Objectives: Many lymphocytic responses in immunity are decreased with age. This decrease occurs with a normal number of T or B cells being present in the lymphoid organs and, therefore, must reflect a decrease in the number in a population which are functional or could reflect a decrease in the division potential of the population. This project is concerned with very early events in an in vitro lymphocyte response with a quantitative assay for triggered T and B cells.

Methods Employed: The assay makes use of the fact that lymphocytes which are resting are impervious to infection with virus, while a lymphocyte which is triggered by a mitogenic stimulus can be infected. Therefore, the assay is for the number of infected cells which is determined by using lytic virus and distributing the lymphoid cells on a monolayer and determining the number of plaques which appear. Since interferon can inhibit viral infection, it must be considered as a part of the assay. Interferon can be measured by determining the inhibition of viral RNA synthesis using a radiolabeled RNA precursor. Further studies are concerned with methods to determine the changes in a lymphocyte membrane which allows the cell to be infected. These methods are standard metabolic assays for the turnover of membrane glycoprotein and lipoprotein along with membrane associated enzyme assays for cyclic nucleotide synthesis.

Major Findings: The viral plaque assay proved to be useful for quantitating the cellular response to mitogen stimulation. The assay, however, was limited to the analysis of the early events in the culture because it was found that interferon was elaborated at later periods and this interfered with the assay system. It was also found that the amount of interferon produced by spleen cells was dependent on the age of the cell donor. Old mice had cells which produced ten times the amount of interferon as did cells from young mice. The effect of interferon on thymidine incorporation was also studied and was shown to be a dose-related inhibitory effect. Therefore, it would appear that the cells from old mice respond poorly to mitogens for at least two reasons: one, there are fewer responsive units, and, two, there are inhibitory substances elaborated by the cells which prevent cell proliferation.

Significance to Biomedical Research and the Program of the Institute: The causes of the increase in interferon elaboration by cells from old mice may yield insight into age-related pathologic mechanisms.

Proposed Course: Terminated.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF</b> INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01-AG-00101-01-LCP												
PERIOD COVERED July 1, 1976 to September 30, 1976														
TITLE OF PROJECT (80 characters or less)  Relation between Nutritional State and Aging														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 30%;">C. H. Barrows</td> <td style="width: 30%;">Acting Chief, LCCP</td> <td style="width: 25%;">LCP NIA</td> </tr> <tr> <td>Other:</td> <td>J. E. Johnson, Jr.</td> <td>Staff Fellow</td> <td>LCP NIA (EOD 6/20/77)</td> </tr> <tr> <td></td> <td>P. L. Mann</td> <td>Visiting Fellow</td> <td>LCP NIA</td> </tr> </table>			PI:	C. H. Barrows	Acting Chief, LCCP	LCP NIA	Other:	J. E. Johnson, Jr.	Staff Fellow	LCP NIA (EOD 6/20/77)		P. L. Mann	Visiting Fellow	LCP NIA
PI:	C. H. Barrows	Acting Chief, LCCP	LCP NIA											
Other:	J. E. Johnson, Jr.	Staff Fellow	LCP NIA (EOD 6/20/77)											
	P. L. Mann	Visiting Fellow	LCP NIA											
COOPERATING UNITS (if any)  None														
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Comparative Physiology														
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224														
TOTAL MANYEARS: 5.55	PROFESSIONAL: 1.55	OTHER: 4.0												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords)  Two dietary regimens have been offered to <u>young-growing</u> and <u>adult</u> animals to determine whether a common, unique, <u>biochemical alteration</u> could be responsible for the reported <u>increase in the life span</u> of rodents. The variables measured in various tissues were cholinesterase, succinoxidase, malic dehydrogenase, DNA and protein.														

## Project Description:

Objectives: It is of importance to establish whether various dietary manipulations which increase lifespan do so by a common biological mechanism. Therefore, efforts were made to determine whether this mechanism is expressed as a biochemical alteration common to those animals offered the various dietary regimens. The biochemical variables measured were protein, DNA, and various enzymes in the tissues of growing and adult mice.

Methods Employed: Animals were fed by one of the following methods: 1) a diet containing 24% protein fed either ad libitum or intermittently (Monday, Wednesday, and Friday) and 2) a diet containing 8% or 4% protein fed ad libitum. Succinoxidase, cholinesterase, malic dehydrogenase, DNA, and protein were determined by standard biochemical procedures.

Major Findings: These data demonstrate that cells, estimated by DNA, of the liver and kidney of growing animals fed low dietary protein (4%) are lower in total protein as well as selected enzymes as compared to those of animals fed a 24% protein diet. These findings agree with our previous reports in which low enzyme levels had been taken as an expression of reduced protein synthesis in animals fed a low protein diet. This conclusion was based on the assumption that the animals were in a steady state in relation to protein synthesis. In contrast, these variables in the cells of growing animals, intermittently fed but sacrificed after 24 hour feeding, were higher than those of normal animals. In order to be assured to what extent the values obtained following feeding were representative of a "steady state" in respect to protein synthesis, animals were also sacrificed following a 24 hour fasting period. These data indicated that enzymatic activities of intermittently fed animals ranged from that observed in normal animals to significantly higher levels. Essentially the same findings were obtained in adult animals. Therefore, at present, enzymatic activity per unit DNA may not be taken as an estimate of steady state levels of protein synthesis in intermittently dietarily restricted animals. Furthermore, they indicate the necessity for a direct measurement of protein turnover by radioisotopic techniques in dietarily restricted animals. Finally, these data fail to identify a common biochemical characteristic among animals fed diets reported to increase lifespan.

Significance to Biomedical Research and the Program of the Institute: The maintenance of health and vitality throughout the lifespan and especially in later years has long been sought. Nutrition is an environmental factor which is easy to manipulate. Therefore these studies are important to determine the degree to which the deleterious effects of aging can be delayed or eliminated by simple dietary methods.

Proposed Course: To date our results have supported a working hypothesis that increased lifespan associated with reduced dietary protein intake may be related to the rate of genetic informational transfer. Attempts will be made to confirm this hypothesis by measuring the rates of turnover of various tissue proteins in these animals.

Earlier studies had suggested that the increased longevity shown by dietarily restricted animals may have resulted from alterations in the pituitary gland. Experiments have recently been initiated to compare anatomical differences in this organ as well as endocrine glands under its control by the use of electron microscopy.

Data presently available suggest that age in man is accompanied by reduced levels of various vitamins in plasma. It is essential to determine the degree to which these observations result as a consequence of decreased dietary intake or impaired absorption by the elderly. Therefore, future studies will include assessment of absorption of various nutrients in animals as well as man.

#### Publications:

Barrows, C.H. and Kokkonen, G.C.: Protein synthesis, development, growth and life span. Growth 39: 525-533, 1975.

Barrows, C.H. and Kokkonen, G.C.: Nutrition and Senescence. In Rechcigiel, M. (Ed.): Handbook of Nutrition and Food. Cleveland, CRC Press, in press.

Barrows, C.H. and Kokkonen, G.C.: Relation between nutrition and aging. Adv. Nutrit. Res., in press.

Barrows, C.H. and Roeder, L.M.: Nutrition. In Finch, C.E. and Hayflick, L. (Eds.): Handbook of the Biology of Aging. New York, Van Nostrand Reinhold Co., 1977, pp. 561-581.

Leto, S., Kokkonen, G.C., and Barrows, C.H., Jr.: Dietary protein, life-span, and biochemical variables in female mice. J. Gerontol. 31: 144-148, 1976.

Leto, S., Kokkonen, G.C., and Barrows, C.H., Jr.: Dietary protein, life-span, and physiological variables in female mice. J. Gerontol. 31: 149-154, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01-AG-00102-01-LCP
PERIOD COVERED July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (80 characters or less)  Age-dependent Stability of the Differentiated State of the Mammalian Cell		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: R. G. Cutler Other: T. Ono	Research Chemist Visiting Fellow	LCP NIA LCP NIA (EOD 10/1/76)
COOPERATING UNITS (if any)  None		
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Comparative Physiology		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS: 1.8	PROFESSIONAL: 1.5	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  This project is designed to determine the long-term stability in terms of specific gene expression of specific types of <u>differentiated mammalian cells</u> . It appears possible that much of the <u>aging process</u> (the increased frequency of diseases and physiological dysfunctions) could result from cells slowly losing their normal highly specialized differentiated state. This would be manifested by either <u>derepression and/or repression of genes</u> that are not normally expressed or essential for cell function. Such behavior could clearly lower the operating efficiency of a cell and could even transform cells leading to certain types of <u>cancer</u> . We have therefore investigated the possible derepression of <u>globin and C-type virus genes in brain and liver</u> cells as a function of increasing age in mice. Our results show up to a two-fold increase in the transcriptional expression of these genes with increasing age.		

## Project Description:

**Objectives:** The proper function of the adult mammalian organism is dependent upon maintaining the differentiated stability of many different types of cells. Any deviation from the optimum differentiated state of a sufficient number of cells is likely to have serious effects on the functional efficiency and health status of the organism. This deviation could decrease in the operating efficiency of the entire organism and give rise to specific types of disease processes. Our objective is to examine the possibility that the aging process of mammals, as well as the age-dependent increase in frequency of many different types of diseases, may be the result of a slow loss in the ability of cells to maintain their differentiated state. The method used is to look for the expression of genes in cells as a function of increasing age of the animal that are not expected to normally be expressed.

**Methods Employed:** Two types of genes are now being investigated. These are  $\alpha$  and  $\beta$  globin genes and the C-type virus endogenous genes that have been shown to cause leukemia of the mouse. The presence of RNA sequences transcribed from these genes are detected by using a complementary DNA probe (c-DNA). This probe is obtained by using  $\alpha$  and  $\beta$  globin and C-type virus RNA as a template to synthesize a highly radioactive c-DNA molecule using reverse RNA-polymerase. The method is able to detect less than five molecules of RNA transcribed by these genes per cell. RNA from nuclei and cytoplasm of liver and brain cells are extracted as a function of increasing age from C57Bl/6J male mice and these RNA preparations are then examined for the presence of the  $\alpha$  and  $\beta$  globin and C-type virus RNA sequences.

**Major Findings:** An increase in the amount of RNA complementary to both the  $\alpha$  and  $\beta$  globin c-DNA probe and the C-type virus c-DNA probe was found in the nuclei and cytoplasm from both liver and brain cells with increasing age of the mouse. Up to a two-fold increase was found for both nucleic and cytoplasmic RNA preparations over an age range of 6 to 24 months. The results of the  $\alpha$  and  $\beta$  globin, as shown by control experiments, could not be accounted for by a possible contamination of our RNA preparations by reticulocyte RNA.

**Significance to Biomedical Research and the Program of the Institute:** The expression of globin genes in neurons and adult liver tissue plus the increase of this expression in older animals is clearly an unexpected finding and supports the hypothesis that the ability of cells to maintain a differentiated state does decrease with increasing age. Moreover, the age-dependent derepression found for the C-type viruses which have been indicated as a causative factor in leukemia suggests that a general decrease in the ability of cells to maintain the repressed state of many other endogenous virus genes may be occurring with increasing age. These results, if confirmed by more extensive studies, could lead to a further understanding of major cellular changes that underlie the general aging process of man and the increased onset frequency of some types of cancers.

Proposed Course: A more extensive study is now being undertaken to further investigate the derepression of  $\alpha$  and  $\beta$  globin and C-type virus genes in mouse tissues as a function of age. This involves the isolation of specific types of brain and liver cells and the inclusion of more animals in the intermediate age ranges. In addition, we are preparing globin and C-type virus c-DNA probes specific to human cells in order to investigate whether a corresponding age-dependent derepression also occurs in man.

Publications:

Cutler, R.G.: Evolution Biology of Senescence. In Behnke, J.A., Finch, C.E. and Moment, G.B. (Eds.): Biology of Aging, in press.

Cutler, R.G.: A New Look at Biological Aging: An Overview. In Behnke, J.A., Finch, C.E. and Moment, G.B. (Eds.): Biology of Aging, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF          INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER  Z01-AG-00103-01-LCP								
PERIOD COVERED <p style="text-align: center;">July 1, 1976 to September 30, 1977</p>										
TITLE OF PROJECT (80 characters or less)  <p style="text-align: center;">Age-dependent Physico-chemical Characterization of Chromatin in Mammals</p>										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">R. G. Cutler</td> <td style="width: 30%;">Research Chemist</td> <td style="width: 20%;">LCP NIA</td> </tr> <tr> <td>Other:</td> <td>R. G. Dean</td> <td>Staff Fellow</td> <td>LCP NIA</td> </tr> </table>			PI:	R. G. Cutler	Research Chemist	LCP NIA	Other:	R. G. Dean	Staff Fellow	LCP NIA
PI:	R. G. Cutler	Research Chemist	LCP NIA							
Other:	R. G. Dean	Staff Fellow	LCP NIA							
COOPERATING UNITS (if any)  <p style="text-align: center;">None</p>										
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Comparative Physiology										
INSTITUTE AND LOCATION <p style="text-align: center;">NIA, NIH, Baltimore, Maryland 21224</p>										
TOTAL MANYEARS: <p style="text-align: center;">1.9</p>	PROFESSIONAL: <p style="text-align: center;">1.5</p>	OTHER: <p style="text-align: center;">0.4</p>								
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords)  <p>           This study was initiated to characterize the <u>physico-chemical</u> properties of the chromatin taken from <u>specific cell populations</u> of cells of animals throughout their lifespan. This information is necessary to determine if alterations of the chromatin could play an important role in <u>underlining</u> the general <u>aging process</u> of the animal. Our results indicate that significant changes in the binding properties of chromatin proteins to DNA do occur with increasing age in mice. However, reports from other laboratories that a significant amount of <u>single-stranded</u> DNA also accumulates with age were not confirmed.         </p>										



## Project Description:

Objectives: The general aim of this research project is to determine if primary aging processes exist at the cellular level that might underlie many of the complex physiological changes that are characteristic of the mammalian aging process. The specific project now underway is designed to investigate whether the genetic material of cells remains intact throughout the lifespan of the animal or if significant physico-chemical alteration occurs that could lead to the improper function of the cell and possibly to the aging of the organism.

Methods Employed: The major genetic material of mammalian cells consists of a DNA-protein complex called chromatin. This chromatin is found in the nucleus of the cells. DNA-DNA, DNA-protein and protein-protein complexes of a covalent nature are not normally found in chromatin but could be caused by a number of mutagenic and/or carcinogenic agents as well as many other natural by-products of metabolism that exist in a cell. Although the cell is likely to have repair and protective processes to lower the damage rate of chromatin, the accumulation of such damage could have far reaching consequences to the proper function of the cell and organism.

We have developed techniques to detect the type and amount of DNA-protein complexes in chromatin as a function of age in C57Bl/6J male mice. The major techniques used were (1) the extractability of chromatin proteins from DNA, (2) the presence and stability of DNA-protein complexes as detected by a nitrocellulose membrane filter assay and (3) the thermal stability of native chromatin complexes.

There has also been a number of reports from other laboratories indicating a significant age-dependent accumulation of single-stranded regions of DNA occurring in mouse liver and brain tissue. Values up to 20% single-strandedness were reported to accumulate over the lifespan of the mouse. Such a high degree of single-stranded regions as found in old mice could easily interfere with the proper function of the cell and so we attempted to confirm this finding in our laboratory. The method used to detect these single-stranded regions was the sensitivity of the purified DNA preparations to  $S_1$  nuclease digestion. This enzyme acts specifically to single-stranded regions of DNA.

## Major Findings:

(1) The extraction efficiency of chromatin proteins (histones and non-histones) was found to decrease with increasing age of mouse liver and brain tissues.

(2) An increase in the amount of DNA-protein complexes was also found with increasing age using the nitrocellulose membrane filter assay.

(3) No changes were found in the thermal stability of whole chromatin preparations.

(4) No significant amount of single-stranded DNA or its accumulation with increasing age was found.

Significance to Biomedical Research and the Program of the Institute:

The results of these studies are essential to our understanding of the biochemical nature and origin of the aging process of man. In this respect it is of primary importance to determine if the cells of man become damaged as a function of increasing age. The experiments reported here indicate that chromatin alterations do occur and accumulate with increasing age in brain and liver cells of mice. Whether or not this type of change acts in a causative manner in the aging process of mice and also occurs and plays this role in man remains to be determined.

Proposed Course: A more detailed study is now underway to determine the specific biochemical nature of the physico-chemical alteration that had been found in chromatin. We plan on further developing our assay techniques to increase their sensitivities. We also plan to utilize a high-pressure liquid chromatography system to detect and resolve some of the possible DNA-protein adducts that might exist in the chromatin of older mice. These studies will also be extended to determine what effects the chromatin alterations found in older mice have on the transcription activity of a cell and its effect on the ability of cells to maintain their differentiated state of function. These studies will utilize specific types of cell populations taken from liver and human tissues.

Publications:

Cutler, R.G.: Alterations with Age in the Informational Storage and Flow Systems of the Mammalian Cell. In Harrison, D.E. (Ed.): Genetic Effects of Aging. New York, A. R. Liss, Inc., in press.

Gaubatz, J.W. and Cutler, R.G.: Age-related differences in the number of ribosomal RNA genes of mouse tissues. Gerontology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF          INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER  Z01-AG-00104-01-LCP
PERIOD COVERED July 1, 1976 to September 30, 1977		
TITLE OF PROJECT (80 characters or less)  Clinical Immune Survey of the Longitudinal Project Participants		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:  Other:	W. H. Adler A. A. Nordin K. H. Jones J. E. Nagel P. L. Mann S. J. Hausman M. A. Brock	Medical Officer, PHS Research Chemist Staff Fellow Clinical Associate, PHS Visiting Fellow Staff Fellow Research Biologist           LCP NIA LCP NIA LCP NIA LCP NIA (EOD 7/1/77) LCP NIA LCP NIA LCP NIA
COOPERATING UNITS (if any)  None		
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Comparative Physiology		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS:  1.6	PROFESSIONAL:  1.2	OTHER:  0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This project was initiated in July, 1977, and the data is insufficient for analysis at this time; the majority of the time so far has been devoted to the development of methods for assay systems. The goal of this project is to gain an overall picture of individuals' <u>immunologic ability</u> by assessing different <u>immune functions</u> and correlating the results. The three broad areas of this program will be data collection, research and service. The data collection and research aspects will be dealt with specifically in terms of investigation of <u>serum antibody</u> and <u>lymphocyte responses</u> . The service aspect will be in terms of a consultative function in setting up assays that use immunological methods, such as <u>radioimmune</u> assays for <u>hormones</u> , or <u>fluorescent antibody staining</u> in histopathology.		









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